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CONTENTS

CHAPTER 1

BANANA BREEDING STRATEGIES FROM PAST TO PRESENT

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Diversity of Banana Groups

Banana, an indispensable product of tropical and subtropical climates, is the motherland of Indochina and South-East Asia. It is thought that there are more than fifty wild species of banana. *Musa acuminata* and *Musa balbisiana* are responsible for the cultivation of most edible banana species (CGIAR, 2022). Wild bananas are diploid and inedible because their fruits are seeded. Most cultivated bananas are triploid. Triploid varieties are more vigorous and productive and have now replaced the rare diploid varieties. The main purpose of banana breeding is the recombination of biologically sterile triploid varieties with fertile diploid genotypes to meet the producer and consumer demands. Although some significant progress has been made in banana cultivar breeding in the last twenty years, only several hybrid cultivars (many of them are tetraploids) are cultivated in important areas. When a grouping is made according to different genome structures and numbers, a list as follows was created (Table 1). The list includes the names of some known cultivars belonging to groups and subgroups.

Groups, Subgroups, Cultivars	**Diversities**
AA genome group	It includes all diploids arising from *Musa acuminata*. Diploid cultivars are edible.
AB genome group	It includes all varieties with two sets of chromosomes transmitted by A-*M. acuminata* and B-*Musa balbisiana*.
AAA genome group • Cavendish subgroup (Pei Chiao, Giant Cavendish, Bungulan, Grande Naine, Williams) • East African highland banana subgroup (Mbwazirume) • Gros Michel subgroup (Gros Michel, Bogoya)	Cavendish cultivars have three sets of chromosomes from the wild *M. acuminata* species. Triploid East African Highland bananas are a subgroup of cooking and beer bananas. The distinguishing feature of Gros Michel, similar to the Cavendish cultivars, is the green or pale pink color of the pseudostem sheath.
AAB genome group • Iholena subgroup (Gerei Langi, Iholena lele) • Maoli-Popoulu subgroup (Maoli Maoli, Huamoa) • Mysore subgroup (Mysore) • Plantain subgroup (Obino l'Ewai, Apantu) • Pome subgroup (Lady finger) • Silk subgroup (Latundan)	All the cultivars have two *M. acuminata* and one *M. balbisiana* chromosome sets. Some cultivars are like plantains and some are dessert bananas.

ABB genome group • Bluggoe subgroup	All the cultivars have one set of *M. acuminata* and two sets of *M. balbisiana* chromosomes. The cultivars used primarily for cooking
Fei bananas (Asupina, Karat)	Fei banana is cultured independently from the bananas related to *M. acuminata* and *M. balbisiana*. The bunch structure is known to be erect.

Table 1. List of banana groups and subgroups (Simmonds & Shepherd, 1955)

The main purposes of banana breeding are as follows:

1. Development of dwarf and semi-dwarf statured varieties suitable for high density planting and prevention of high winds.

2. Development of long and high-quality fruiting varieties suitable for export.

3. Development of varieties resistant to biotic and abiotic stress factors (such as nematodes, bunchy top, *Fusarium* wilt, Moko, Sigatoka, and pseudostem weevil).

4. Development of new varieties with wider agro-ecological adaptability.

This study is intended to show how much progress has been made in banana breeding so far.

1. Conventional Breeding

Few qualitative or quantitative traits have been revealed in 80 years of classical banana breeding. Through cultivation, various morphological and physiological features have been used to distinguish domesticated products from their wild ancestors (Poncet et al., 1998). For thousands of years, people's priority for edible bananas was to choose plants whose fruits were seedless. However, as time passed, developing bananas for disease resistance, tolerance to environmental stress, and desired post-harvest quality has become a newer activity.

Producing a new generation requires seeds. For bananas, even if seeds are produced, very few germinate and therefore seed production and germination are a problem in banana breeding. Wild banana seeds germinate very quickly while still fresh, and as they dry they go into physical dormancy. Most banana seeds do not germinate as they do not contain embryos. The continuity of genetic inheritance in bananas continues only with the seed. For this, pollination is necessary. Pollen can come from the same flower, another flowers of the same genotypes (autogamy), or another gen-

otypes (allogamy). This mechanism ensures genetic recombination while maintaining variability within populations at all times (Tenkouano et al., 2011)

The banana breeding studies became essential after the break out of the *Fusarium oxysporum f.sp. cubense* tropical race 4 (*F*. wilt TR4). The studies were first initiated in Panama in 1920, in Trinidad in 1922, and in Jamaica in 1924 (Shepherd, 1994). In later times, banana breeding continued in Brazil (Shepherd et al., 1992), in Guadeloupe (Bakry & Horry, 1993), in Honduras (Rowe and Rosales, 1993), in India (Sathiamoorthy and Balamohan, 1993), and in Nigeria (Vuylsteke et al., 1993; 1997).

Triploid AAA bananas emerged from *M. acuminata* (AA) and natural hybridizations with *M. balbisiana* (BB) resulted in the formation of hybrids (plantains and bananas (AAB), and cooking bananas (ABB)). When distinguishing these hybrids from each other, descriptive criteria such as the presence of male bud-flower, hermaphrodite flowers, pseudostem coloration-blotching, leaf direction, and waxiness were taken into account (Ortiz, 2013). The quantitative descriptors like pseudostem circumference, pseudostem height, fruit number, and fruit length have a high heritability and repeatability while they have a low coefficient of variation. Understanding the relationships between the growth and development characteristics of bananas is very important for the breeding of new varieties. Aguilar Morán (2011) reported that the bananas of the Cavendish group are female sterile, and the development of new cultivars is dependent on 'Gros Michel' and its short version mutant 'Highgate' cultivar. According to Stover and Simmonds (1987), no seed formation was observed in crosses made within the Cavendish group, but 'Gros Michel' bananas were able to produce one or two seeds per bunch.

Many banana fruits have varying levels of female sterility genes. In addition, the chromosome structure and the nature of triploidy should be investigated in detail in all cultivars. In cultivar development studies, seed development depends not only on the mother but also on the availability of pollen (Simmonds, 1962). To determine the male fertility of 'Cavendish' bananas, 'Calcutta IV', a diploid wild cultivar, was used as the female parent. After crossing 'Calcutta IV' with a 'Cavendish' cultivar, 1500-2000 seeds per bunch were obtained. This was the first report to show that 'Cavendish' bananas are not male-sterile (Aguilar Morán, 2011).

IITA and FHIA have developed new diploid genotypes with different levels of disease resistance (Tenkouano et al. 2003; Rowe & Rosales, 1993). For example, the diploid 'Calcutta IV' variety is resistant to many diseases (Sigatoka, *F.* wilt, weevil, and nematodes) (Ortiz, 2015). New diploid cultivars were obtained by using this cultivar. This allowed the trans-

fer of new traits with crossbreeding studies. Figure 1 is a schematic of what a crossbreeding study would look like:

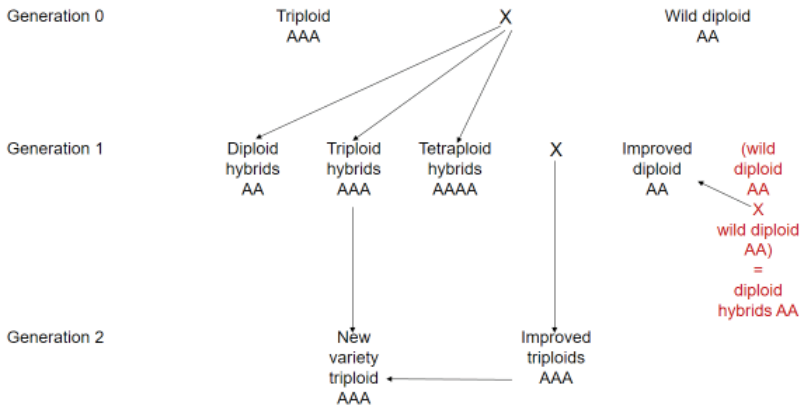

Figure 1. *The process of conventional banana breeding*

Crossbreeding in bananas is done by hand in the early morning. However, it takes a long time, such as a few months, to obtain seeds after hybridization. It has been reported to be in the range of 0.3-21.70 seeds per bunch (Swennen & Vuylsteke, 1993). A bunch is harvested before physiological maturity, usually when the distal fingers turn slightly yellow. After the bunches are left to mature, the seeds are immediately removed and prepared for embryo culture to prevent seed/embryo drying (Bakry et al., 2009; Uma et al., 2011).

Ploidy numbers of hybrid individuals can be hyperploid, and aneuploid except diploid, triploid and tetraploid. Ploidy levels were formerly estimated by phenotypic appearance, and root tip mitosis or stomata density, size, and the number of chloroplasts per guard cell pair were examined. Root tip mitosis study is accepted as one of the reliable methods to determine ploidy status in bananas (Kumar, 2006). Krishnamoorthy (2002) performed root tip mitosis in all 36 parthenocarpic hybrids whose stomatal characteristics were evaluated.

In the genus *Musa*, flow cytometry is mostly used in ploidy analysis in recent years (Awoleye et al., 1994; Dolezel et al., 1997; Johnson et al., 1998; Egesi et al., 2002; Emshwiller, 2002; Bonos et al., 2002; Beatson et al., 2003; Walker et al., 2005). Baysal et al. (2022) determined ploidy levels of commercial 'Dwarf Cavendish' and 'Grand Nain'; local 'Erdemli Yerli', 'Azman' and 'Küllü Erdemli Yerli', and wild 'F4' (*Ensete glaucum* the

Yunnan banana), 'F5' (*Ensete ventricosum* the Abyssinian Banana), and F3 (*Ensete glaucum* the Snow Banana) genotypes were determined through flow cytometry. Of the 'F3', 'F4', and 'F5' genotypes obtained from seeds, 'F4' and 'F5' were determined as diploids, while F3 was determined as a spontaneous triploid. Commercial and local cultivars were triploid.

In the new generations obtained from hybrids, different ploidy levels are determined and even sometimes it can contain mixtures of aneuploidy (Brown et al., 2017). Oselebe et al. (2006) reported that in crossing between 2x-2x, hybrid progeny were almost entirely diploid (99.7%), although mixed ploidy hybrids tended to include individuals differing in chromosome set number. When a diploid individual was used as the maternal parent in a 2x-4x hybrid cross, more than 96% of the progeny were diploid. However, when tetraploid was used as the maternal parent (4x-2x), the observed new generation was predominantly triploid. It was also observed with varying degrees of other ploidy levels (94%) between diploid and pentaploid.

Wilson et al. (2020) published the results of their 10-year research on classical breeding bananas. Over 320 types, cultivars, clones, varieties, etc. (2x, 3x, 4x) were collected from different parts of the world and used in the study, and crosses were made by creating different combinations. As a result of the study, the 2x-2x hybrid combination produced 11 times more seeds than the 2x-3x hybrid combination and 54 times more seeds than the 2x-4x hybrid combination. Also, in all combinations, the most seeds were obtained from diploid males. Besides, as the ploidy level of the maternal parent increased from diploid to tetraploid, pollination success increased, especially in diploid males.

While 'Gros Michel' was the dominant variety in banana plantations between 1800 and 1950, Cavendish groups became the dominant variety in the early 1970s. Especially after the outbreak of Panama disease (*F.* wilt TR4), the interest in the Cavendish group cultivars increased, but it was understood that they were sensitive over time. Grim (2008) reported that revealing the banana genome will enable the development of varieties resistant to *(F. wilt TR4)*. The severity of this disease was so huge that new modern techniques were needed to develop new disease-resistant varieties. In 2002, 20 tetraploid hybrids were developed under the Banana and Plantain Breeding Program (FHIA). They pollinated 'Williams' and 'Grand Nain' banana cultivars with the 10 'Cavendish' cultivars. 20 of the 40 embryos that survived were determined as tetraploid. As a result of the study, it has been shown that 'Cavendish' cultivars can be used in crossing studies even if they are low fertile (Aguilar Morán, 2013). However, the necessity of crossbreeding in large numbers (thousands of hybrid combinations) was both a waste of time and a waste of land.

2. Mutation Breeding

Mutation-based approaches are particularly useful in species that have a narrow genetic base and do not conform to conventional breeding methods. Mutagens are physical (cosmic rays, ɣ-rays, x-rays, ultraviolet), chemical (ethyl methanesulfonate (EMS), diethylsulfonate (DES); sulphur mustards; nitrogen mustards, sodium azide, diazomethane, etc.), and biological known as agents (Bradshaw, 2017).

The first mutation studies in bananas were carried out on suckers and seeds. Stotzky et al. (1964) investigated the effects of gamma rays on seed germination and seedling survival in diploid *M. balbisiana*. Azzam and Linden (1965) reported that the suckers of 'Gros Michel' showed reduced germination after irradiation and no surviving plant at doses higher than 50 Gy. Fortuno and Cedeno Maldona (1972) found a mutant with leaf aberrations and another mutant with more intense pigmentation selected in the M_1V_2 generation.

In vitro studies paved the way for mutation studies in bananas. De Guzman et al. (1982) Guzman reported that suckers can be exposed to doses of more than 100 grays. While *in vitro* studies were not yet widespread, sucker irradiation was quite difficult. Because, the number of regenerated suckers was low in the M_1V_1 generation. Today, tissue culture applications are widely used in mutation breeding. In Figure 2, mutation breeding steps are briefly given.

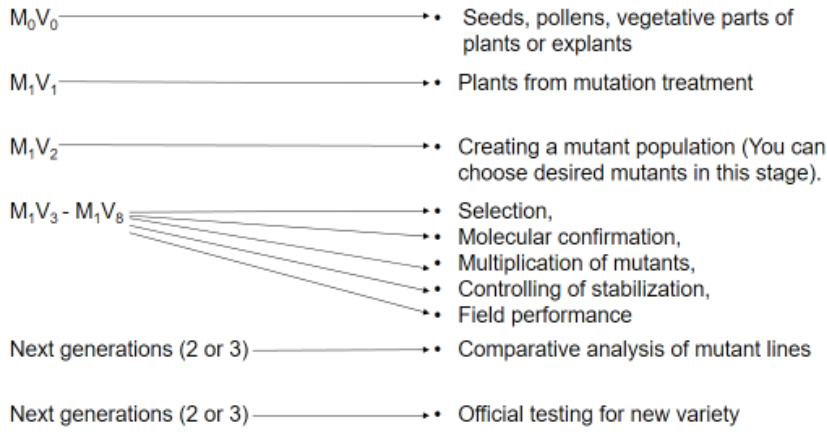

Figure 2. *The stages of mutation breeding (M: meiotic, V: vegetative generation)*

Hundreds of mutation breeding studies have been carried out in bananas so far. Particularly, physical and chemical mutagen applications are used intensively in banana breeding as in many species. Some characteristics have been given to banana plants by mutation breeding. Gamma rays were applied to 'Dwarf Cavendish' and 'Williams' banana cultivars and determined mutant individuals tolerant of *(F. wilt TR4)*. Besides, mutant plants were more cold tolerant and grow faster (Smith et al., 1995). The finding of genotypes that are predominantly tolerant to diseases and some stress factors through mutation breeding in bananas has increased the number of these studies.

Dwarfism is a desirable trait in banana cultivation and breeding because semi-dwarf varieties resist damage from wind and rain well. Smith et al. (2006) obtained mutant individuals from an extra-dwarf variety, 'Dwarf Parfitt', and determined that the mutants were between 'Williams' and 'Dwarf Cavendish' banana varieties. They also reported that the mutants were colder tolerant and had a faster growth cycle than the 'Williams' variety. Chen et al. (2016) obtained the dwarf mutant '8818-1' from the 'Williams-8818' banana cultivar by EMS (ethyl methane sulfonate) mutagenesis. They reported that the content of gibberellins (GAs) in the pseudostem of '8818-1' was significantly lower than that of its parent, '8818', and the dwarf type of '8818-1' could be recovered by external application of GA_3. Novak et al. (1995) detected changes in morphological (plant height, leaf shape) and physiological (bottom shoot growth, proliferation, flowering time, fruit ripening) and agronomic (mottle diameter) characters in plants after physical and chemical mutagen application. Ramesh Kumar et al. (2008) determined two nematodes resistant and one moderately resistant mutant as a result of their mutation breeding study in 'Rasthali' and 'Robusta' banana cultivars. Xu et al. (2006) identified mutant individuals with trichomes (hairs) on the fruit epidermis, unlike the plant and fruit stem, in the mutant individual obtained from the 'Williams' banana cultivar. Nettyani and Dan Sobir (2014) detected mutant individuals without morphological male buds in their mutation breeding in the 'Kepok' banana cultivar. Zou et al. (2015) reported that the pseudostem of the mutant 'Qinggan' cultivar they obtained from the 'Guijao' banana cultivar was green compared to the mother plant and it was an early maturing cultivar.

Physical mutagens and chemical mutagens provide great benefits in practice in banana breeding. In this regard, Valerin et al. (1995) applied the chemical mutagen EMS (ethyl methanesulfonate) to 50 banana clones and obtained 1600 plants. Mutant plants with different structures were obtained in terms of leaf shape, color, and distribution in plants. Of these, 128 plants responded differently to Black Sigatoka disease, and nine were determined to be tolerant. Besides, dwarfism was observed in plants. Roux

et al. (2007) established a mutant population from the 'Williams' cultivar of 4000 plants. They determined that 15 plants assumed to be mutants were tolerant to *Mycosphaerella fijiensis* disease.

When the morphological observations were made in plants, Imelda et al. (2001) detected differences in leaf color, pseudostem height, and formation of two pseudostems from one main pseudostem in only 31 of 605 mutant individuals. They reported that many differences in other individuals were detected with electrophoretic enzyme samples, but not with morphological or phenotypic observations. Ganapathi et al. (2008) reported that gamma-ray provided dwarfism in the phenotype in their study on the 'Giant Cavendish' banana cultivar, and the yield was comparable to control plants. Mahdi et al. (2014) morphologically compared mutant Williams genotypes with classical 'Dwarf Cavendish' and 'Williams' cultivars. Mutants and the original 'Williams' variety had the longest pseudostem length, while 'Dwarf Cavendish' and mutant 'W-193/3' had the smallest height. As a result of the study, it was understood that the mutants had a stronger growth force than the others and especially 'W-193/3' had a short growth period. Similarly, Mak et al. (1996) obtained 27 mutant individuals from the 'GN-60A' (Grand Nain) genotype as a result of mutation breeding studies. Twenty-two individuals with high yields of early blooming were reproduced under *in vitro* conditions and transferred to the field. The mutants were compared with the control 'Grand Nain' and 'Williams' varieties. As a result of the study, it was determined that the mutant named 'Novaria' bloomed 10 weeks before the 'Grand Nain' variety. However, no difference was found in terms of bunch weight, hand weight, finger weight, and finger length parameters.

3. In Vitro Technologies

Somaclonal variation is called variation among banana plants regenerated from tissue culture. Somaclonal variations have all been reported in different plant species such as isoenzymes, plant yield, ploidy, disease resistance, etc. (Patil & Navale, 2000). Hwang and Tang (1996) reported that off-type occurrences in plantlets obtained by tissue culture in 'Cavendish' cultivars ranged from 6% to 38%. They also identified 29 examples of somaclonal variation in various types of bananas and plantains. While Bairu et al. (2006) detected somaclonal variations in the fourth cycle (3%), Damasco et al. (1998) also defined variations in the fifth cycle. Reuveni et al. (1993) also determined variations for non-constant families in first-generation replications.

Banana breeding programs are very problematic due to the sterile nature of bananas and almost no seeds can be obtained from the hybrids. The ancestors of edible bananas were healthy and capable of producing seeds. Embryo

culture is a potential tool for the preservation and maintenance of hybrid germplasm. Dayanari et al. (2014) presented the results of germination and regeneration of *Musa ornata* seeds after applications of embryo culture and embryo rescue. They reported that zygotic embryos of *Musa ornata* could be recovered from calli when harvested at a maturity level of 80%. Onganga et al. (2020) reported that the seeds of the genotypes they studied, belonging to *Musa balbisiana*, germinated well with an average rate of 80%, while they reported that different *Musa acuminata* varieties germinated poorly. They attributed this situation to dormancy factors in seeds.

Young male flowers of bananas are used during somatic embryogenesis and plant regeneration. Escalant et al. (1994) achieved the proliferation of somatic embryos from the cultivar 'Grande Naine' by using embryogenic cultures. Morais et al. (2016) obtained regenerated plants from embryogenic cultures using young male flowers of 'Grand Nain' (AAA) and 'Tropical' (AAAB) banana cultivars. However, no genetic variation could be detected among regenerating plants in an evaluation using the SSR technique.

Somatic hybridization by using protoplast fusion method is very important for breeding. Disease-resistance genes are especially found in diploids (AA). However, the transfer of these characters to triploid bananas is extremely difficult using conventional breeding methods. Matsumoto and Oka (1997) published a report about protoplasts. A somatic hybridization was made between triploid and diploid bananas by Matsumoto et al. (2002). Protoplasts from embryogenic cell suspensions ('Maça' (AAB group)) were fused with protoplasts from nonembryogenic calli of the 'Lidi' (AA group) banana cultivar. When the fusion-treated protoplasts were cultured, they observed a clear somatic embryogenesis. After molecular evaluation, 11 out of 13 plants were identified as somatic hybrids. To develop varieties resistant to *(F. wilt TR4)*, Xiao et al. (2009) obtained somatic hybrids after their fusion study from disease-resistant *M. acuminata* cv. Mas (AA) to *Musa silk* cv. 'Guoshanxiang' (AAB). It was determined that the three hybrids had an aneuploid chromosome number (2n = 34).

4. Molecular Assisted Breeding

The common use of MAS (marker-assisted selection) in ongoing banana breeding programs has not yet been achieved. The use of molecular markers for the choice of cultivars under development accelerates the selection process as it is faster than the results of morphological observations. Molecular markers were first used to reveal the genetic differences of individuals in banana breeding and are still used routinely in breeding programs. Numerous molecular studies have been carried out on the *Musa* species so far (Gawel & Jarret, 1991; Gawel et al., 1992; Ude et

al., 2002; Wong et al., 2002; Jarret et al., 1992; Jarret & Gawel., 1995; Shepherd, 1999; Nwakanma et al., 2003; Bartos et al., 2005; Heslop-Harrison & Schwarzacher, 2007; Li et al., 2010; Liu et al., 2010; Nayar, 2010; Christelova et al., 2011; Hřibová et al., 2011). To determine the difference between genotypes, Random Amplified Polymorphic DNA (RAPD) (Das et al., 2009), Restriction Fragment Length Polymorphism (RFLP) (Hippolyte et al., 2010), Amplified Fragment Length Polymorphism (AFLP) (Opara et al., 2010), Sequence Related Amplified Polymorphism (SRAP) (Baysal & Ercisli, 2022) and Simple Sequence Repeats (SSR) (Baysal et al., 2022) markers have been used.

Banana characters arise as a result of more than one genetic trait, none of which is determinative on its own. This condition is called oligogenic epistatic basis. To give example of this situation, parthenocarpy is the most typical example. The post-crossing evaluation found that about half of the several thousand secondary triploid hybrids established each year were non-parthenocarpic. Moreover, a candidate SSRLP marker has been identified for the early detection of parthenocarpy (Crouch et al. 1996). Tetraploid bananas have a shorter flowering period than others. Molecular markers for this situation were determined by Crouch et al. (1998).

DNA markers for many important characteristics, including resistance to pests and diseases in banana cultivars, are still under investigation. Quality parameters such as fruit color, texture, and ripening are also other candidate traits for selection with markers. Lin et al. (2009) reported a method for detecting *(F. wilt TR4)* isolates. National Research Center for Banana (NRCB) identified a RAPD marker for Sigatoka resistance. For salt tolerance identified A RAPD marker tolerance among clones of cv. 'Dwarf Cavendish' (Miri et al. 2009).

5. Genetic Modification and Genome Editing

Genome editing studies for bananas are a next-generation technique used to introduce new traits, such as resistance to viral diseases, into the plant genome. The *Agrobacterium*-mediated transformation method may be more widely applicable. It is dependent on the use of differentiated tissue that can be commonly regenerated into whole plants. Ganapathi et al. (2001) achieved *Agrobacterium*-mediated transformation of embryogenic cell suspensions of banana cv. 'Rasthali' (AAB). Atkinson et al. (2004) developed the first nematode-resistant transgenic banana variety using the *Agrobacterium* method. Pei et al. (2005) created transgenic bananas expressing the human lysozyme gene for Panama wilt resistance.

The first banana gene editing report was about cultivar 'Rasthali' (AAB genome) by targeting the phytoene desaturase (PDS) as a marker

gene and the mutation efficiency was found to be 59% (Kaur et al., 2018). Later, Naim et al published another study in which the mutation efficiency was determined as 100% (Williams banana cultivar, PDS gene). Ntui et al. (2020) also reported 100% mutation efficiency ('Sukali Ndiizi' (AAB genome) banana cultivar and plantain cultivar 'Gonja Manjaya' (AAB genome)). Hu et al. (2022) reduced ethylene synthesis was achieved after upregulation of aminocyclopropane-1-carboxylate oxidase (MaACO1) in bananas. This has shown to extend the shelf life of fruits.

The Baby Boom gene is reported to play an important role as a gene marker in multiple signaling developmental pathways in plant development. Awasthi et al. (2017) identified 8 genes of nine transcription factors families from the banana genome database. Protein sequence analyzes showed the presence of characteristic conserved domains in these transcription factors. They reported that MaBBM2 and MaWUS2 are encouraging candidates for embryogenicity in bananas.

One of the ways to overcome the breeding challenge in bananas is to increase transformation efficiency by using genes that act as morphological regulators, such as Baby boom (Bbm), Wuschel2 (Wus2), and Shoot Meristemless (Stm), which would be a good investment for the future.

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CHAPTER 2

VARIATION OF ANTIOXIDANT ACTIVITY, YIELD, QUALITY, AND FLOUR COLOR IN DURUM WHEAT (*Triticum turgidum* L. var. *durum*) AS A FUNCTION OF CULTIVAR AND NITROGEN

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INTRODUCTION

Wheat is one of the major cereal crops that represent important ingredients for human nourishment because of its universal use for flour, semolina and wheat-based products (bakery, pasta, bulgur, and couscous) (Laus et al., 2012). Durum wheat is a traditional crop that is consumed and included in most dishes in the Mediterranean region where 60% of the world's durum wheat is produced and has ideal climate conditions to produce high yield and suitable quality for durum products (Abad et al. 2004). It is a good source of carbohydrates, protein, dietary fiber, minerals and besides its nutritional value whole grain also contains phytochemicals that have high significant health benefits. Phenolic compounds and antioxidants are the main phytochemicals that directly react with the reactive oxygen species (ROS). These health-benefit chemical compounds have been associated with reduced risk of chronic diseases (diabetes, cardiovascular illnesses, and cancer). The phenolic compounds and antioxidants are primarily concentrated in the outer layers of grain such as aleurone, testa, and pericarp (Martini et al., 2015; Yiğit and Erekul, 2021).

Bioactive compounds spread heterogeneously in the whole grain and grain parts have different antioxidant capacities, the germ, and the bran have the highest phenolic compounds and antioxidant activity compared to endosperm (Yılmaz, 2019). Bran is a key factor in the classification of whole-grain benefits and contains various amounts of phenolic compounds comparing cereals with other plants. Grain structure and milling process (cause different grain fractions and bran separated) lead to high variability in grain structure and antioxidant potential of durum wheat (Călinoiu and Vodnar, 2008; Šramková et al., 2009).

Along with providing healthy compounds durum wheat provides major caloric and protein sources for humanity. Durum wheat quality is highly dependent on protein quality (glutenin and gliadins; provide dough viscoelasticity and extensibility) and quantity (determine nutritional quality). In addition to high protein content, durum wheat contains yellow-colored pigment which is the most desirable wheat quality trait that is preferred in pasta, semolina, and spaghetti production. The color of semolina and pasta changes due to different pigments yellow (preferable, amber color and brown (considered low quality). The carotenoid gives yellow color and ensures end-use quality traits for pasta and semolina (Mazzeo et al., 2017; Saini et al., 2022). Some carotenoids provide protection from chronic diseases which have provitamin A activity and all of them show antioxidant capacity which reduces risk of degenerative diseases (Ficco Donatella et al., 2014). Environmental (i.e climate, stress conditions), agricultural processes (i.e fertilization, pesticides) and genotype factors cause inevasible changes in grain structure and antioxidant content of durum and bread

wheat (Šukolavić et al., 2013; Mpofu, 2016). Nitrogen shortage is one of the main limiting factors of durum wheat and cereals. Proper nitrogen management is essential to ensure higher yield and quality durum wheat production. Design of fertilizer application should combine with rate, timing, and water management with a view of optimizing wheat yield, quality, and nourishment properties (Garrido-Lestache et al., 2005). In the light of the foregoing, this chapter aimed to gather information on the relationship between nitrogen fertilization effects on grain color and antioxidant properties reveal relation with yield, quality, and health benefits of wheat.

MATERIAL AND METHOD

The field experiment was carried out in Aydın Adnan Menderes University, Faculty of Agriculture, Research and Application Farm located about 33 m (37°45′22′′N 27°45′36′′E) above sea level with Mediterranean climate conditions during the 2021 growing season. Totally 4 durum wheat varieties (Çeşit 1252 (Ç 1252), Poyraz, Şölen, Alatay) obtained from Aegean Agricultural Research Institute, İzmir and Field Crops Central Research Institute, Ankara, and 4 nitrogen doses (0, 50, 100 and 150 kg N ha^{-1}) were used in the experiment. The experiment was set up according to the randomized split-plot design with three replications. Plot size and sowing distance between rows were 1.2 x 6 m and 20 cm, respectively. During the growing season nitrogen fertilizer doses were applied as; 0 kg N ha^{-1}, 50 kg N ha^{-1} (before sowing), 100 kg N ha^{-1} (divided into two equal doses-before sowing and at the stage of tillering-Zadoks Growth Scale 21 (ZGS, Zadoks et al. (1974)), 150 kg N ha^{-1} (divided into three doses-before sowing, at the tillering period-ZGS 21, and at the stage of stem elongation-ZGS 31).

Table 1. Soil texture and chemical analysis

Soil Texture[1] (%)	Sand	58.44	SL Sandy-Loam
	Silt	30.23	
	Clay	11.33	
pH[2]		7.75	Slightly alkaline
Organic Matter[3] (%)		0.98	Very low
Methods; [1]: Bouyoucos (1962), [2]: Ayers and Westcot (1989), [3]: Walkley and Black (1934)			

The experimental soil had sandy loam texture with slightly alkaline reaction, and the amount of organic matter was very low (Table 1).

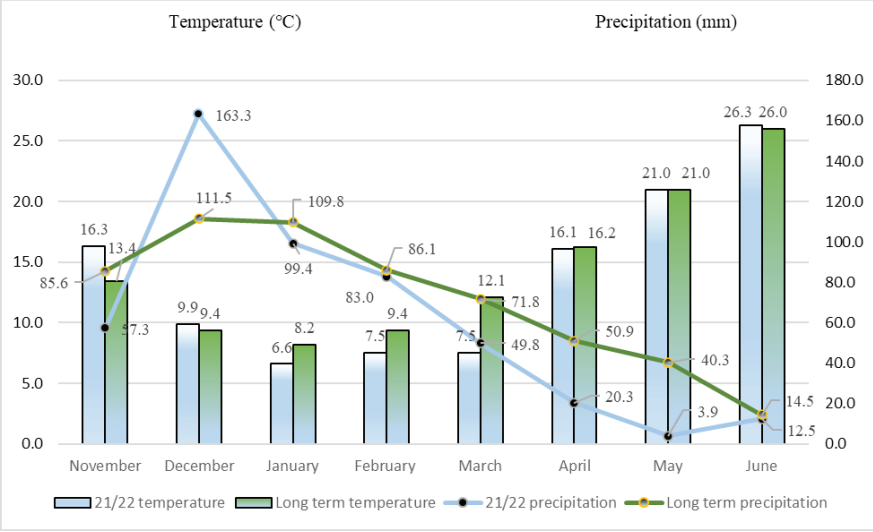

Figure 1. Monthly and long term (1985-2019) climate values

Low-temperature values were observed in January, February, and March, equal to growing stages tillering (from ZGS 21 to ZGS 33) and stem elongation compared to long-term climate values, while temperature values were favorable during generative growth periods. In the early development stages, high precipitation value (163.3 mm) observed in December caused waterlogging conditions during seedling growth stages of durum wheat plants but any adverse effects were observed for crop damage by waterlogging except for delayed stem growth (Figure 1). During the generative growth stages of durum wheat plants, relatively low precipitation values were observed (in April: 50.9-20.3 mm and May: 40.3-3.9 mm) compared to long-term precipitation values. Border lines in each plot were removed from plot at harvesting time, remaining plants were harvested by hand and grains were obtained by ear thresher after that grain yield (kg ha^{-1}) and thousand-grain weight (g) parameters were measured. Durum wheat samples were milled in UDY Corporation, USA miller, and whole durum wheat flour was used in chemical analysis and stored at +4°C until all analyses were completed.

SPAD chlorophyll readings were made using Konica Minolta SPAD 502 device at the beginning of the grain filling period (early milk, ZGS 73). Measurements have performed randomly selected from flag leaves in each plot during a fully-sunny day (Fiorentini et al., 2019).

Near Infrared Reflected Spectroscopy (NIRS) method was used to analyze crude ash, lipid, fiber, and protein content of whole durum wheat flour using Bruker MPA™ (Bruker, Ettlingen, Germany) device. HunterLab ColorFlex EZ, USA color measurement spectrophotometer was used

to determine the flour color of samples by measuring the L* (100=white; 0=black), a*(+ red; - green), b* (+yellow; - blue).

Total phenolic content was determined using Folin-Ciocalteu method (Kaluza et al., 1980; Ragaee et al., 2006) by using gallic acid as standard. Antioxidant activity was performed using modified version of Brand Williams et al. (1995) method that contains the use of free radical DPPH where antioxidants react with the stable radical in methanol solution. Samples (1 g whole flour) were extracted with acidified methanol (HCL/methanol/water, 1: 80/10, v/v) at room temperature for 1 hour in shaker, and obtained supernatant was stored at +4°C until analysis. Statistical analysis of the experimental data was carried out according to the randomized split-plot design with three replications and the differences between the mean values were calculated the LSD multiple comparison method using 'agricole' package (de Mendiburu and de Mendiburu, 2019) in R Studio (V4.1.2). Correlogram was performed in R studio (Boston, MA, USA) by using the 'metan' package (Olivoto and Lúcio, 2020). Heat map was created in R Studio according to the heatmap.2 command within the 'gplots' package (Warnes et. al., 2022).

RESULTS AND DISCUSSION

Yield and quality properties

Durum wheat yield and quality properties may vary widely by many factors during the wheat growing period. Many factors can lead to high variability in yield components, quality, and chemical composition of durum wheat. Nitrogen management is critical and one of the main factors for optimizing yield, protein concentration, and utilization efficiency of fertilizer in durum wheat (Chen et al., 2022). Yield and quality characteristics were examined under different nitrogen doses and durum wheat cultivars. In the study, nitrogen, cultivar and nitrogen x cultivar interaction have been found significant for grain yield, thousand-grain weight, ash and starch content at the level of 0.01. In terms of grain yield, among the cultivars, Poyraz (3629 kg ha^{-1}) and Ç 1252 (3523 kg ha^{-1}) had the highest mean values and Şölen (2565 kg ha^{-1}) cultivar had the lowest value with approx. 30% lower value compared to highest yield value. The response of grain yield varied depending on nitrogen fertilizer doses. High nitrogen fertilization enhanced grain yield approx. 40% compared to control. While grain yield showed a clear response to the N fertilizer dose of the 150 kg ha^{-1}, 50 and 100 kg ha^{-1} exhibited similar responses and particularly caused significant increases compared to control (0 kg ha^{-1}) (Table 2). As reported by Akgün and Ulupınar (2019), the study results points to nitrogen fertilizer application increased the grain yield and the values changed between 1889-4233 kg ha^{-1} although the study results differ slightly increasing the

amount of nitrogen application above 100 kg ha^{-1} did not create a statistically significant increase in grain yield. The grain yield results would seem to show the highest nitrogen fertilizer dose (150 kg ha^{-1}) is probably suggested to achieve high yield values of durum wheat production in Aydın ecological conditions. The cultivars differed in grain yield against different nitrogen dose applications. The single most striking observation that emerged from the data Alatay responded positively to nitrogen increase at 150 kg ha-1 with the highest yield value. Also, statistically significant highest yield values were obtained from Ç 1252 and Poyraz cultivars at 150 kg ha^{-1} compared to other nitrogen dose applications (Figure 2.). The relatively small effect of nitrogen treatment was observed on thousand-grain weight (TGW) in comparison to grain yield. TGW increased due to higher nitrogen fertilization doses compared to non-applied (control) and no significant differences were recorded by increasing fertilizer doses between 50, 100 and 150 kg ha^{-1}. Poyraz (39.2 g) and Alatay (39.3 g) had the highest TGW, whereas Ç 1252 (36.8 g) and Şölen (37.7 g) cultivars had the lowest grain weight (Table 1). A significant interaction between nitrogen fertilization and cultivar was observed and 150 kg ha^{-1} nitrogen dose maximized TGW (47.7 g) of Alatay cultivar approx. 20-40% compared to other cultivars. Alatay responded with linear increasing grain weight to higher fertilization rates. On the other hand, Poyraz cultivar also had the highest TGW (46.6 g) with applied 50 kg ha^{-1} nitrogen dose (Figure 2). The thousand-grain weight results match well with the previous findings (Atar and Kara, 2017; Akgün et al., 2021; Başkonuş et al., 2022).

Table 1. Average values of yield, thousand-grain weight, and SPAD chlorophyll value

		Ç 1252	Poyraz	Şölen	Alatay	Mean nitrogen
GY kg ha^{-1}	0 kg ha^{-1}	2559 f	2994 d	2716 ef	1599 h	2467 C
	50 kg ha^{-1}	3432 c	3697 b	2498 f	2075 g	2926 B
	100 kg ha^{-1}	3514 bc	3386 c	2125 g	2713 ef	2935 B
	150 kg ha^{-1}	4585 a	4440 a	2923 de	4626 a	4143 A
	Mean cultivar	3523 A	3629 A	2565 C	2753 B	
	$Lsd_{Nitrogen}$: 160; $Lsd_{Cultivar}$: 116; $Lsd_{Interaction}$: 238; F $value_{Nitrogen}$: 301*; F $value_{Cultivar}$: 168**; F $value_{Interaction}$: 43**					
		Ç 1252	Poyraz	Şölen	Alatay	Mean nitrogen
TGW g	0 kg ha^{-1}	33.0 fg	32.6 g	36.0 e	34.6 efg	34.1 B
	50 kg ha^{-1}	38.6 cd	46.6 a	39.0 c	36.4 de	40.1 A
	100 kg ha^{-1}	42.0 b	36.5 de	40.5 bc	38.7 cd	39.4 A
	150 kg ha^{-1}	33.5 fg	41.0 bc	35.3 ef	47.7 a	39.4 A
	Mean cultivar	36.8 B	39.2 A	37.7 B	39.3 A	
	$Lsd_{Nitrogen}$: 2.1; $Lsd_{Cultivar}$: 1.0; $Lsd_{Interaction}$: 2.4; F $value_{Nitrogen}$: 62.7**; F $value_{Cultivar}$: 11.9**; F $value_{Interaction}$: 25.2**					
		Ç 1252	Poyraz	Şölen	Alatay	Mean nitrogen
$SPAD_{FL}$	0 kg ha^{-1}	40.4 ef	38.2 fg	32.1 h	40.7 ef	37.8 C
	50 kg ha^{-1}	45.5 c	47.2 bc	42.2 de	36.4 g	42.8 B
	100 kg ha^{-1}	44.0 cd	50.4 a	46.8 bc	50.8 a	48.0 A
	150 kg ha^{-1}	45.7 c	49.2 ab	45.0 cd	51.9 a	47.9 A
	Mean cultivar	43.9	46.2	41.5	44.9	
	$Lsd_{Nitrogen}$: 1.9; $Lsd_{Interaction}$: 3.1; F $value_{Nitrogen}$: 78.8**; F $value_{Cultivar}$: 13.4**; F $value_{Interaction}$: 22.5**					
	GY: Grain yield; TGW: Thousand-grain weight; $SPAD_{FL}$: Flag leaf SPAD value					

SPAD readings are an advanced method and have become common to evaluate leaf chlorophyll content and focused on optimizing N application time in durum wheat, maize, and rice to assess the nitrogen status of plants (Kızılgeçi et al., 2019). The research findings have revealed that the nitrogen rates were significant for SPAD value of durum wheat flag leaf measured in the beginning of the grain filling period. Nitrogen fertilization levels significantly influenced SPAD chlorophyll values of flag leaf. Overall, better results were observed for 100 and 150 kg ha^{-1} nitrogen treatments and these values match with the previous findings in the literature (Fiorentini et al., 2019; Kızılgeçi et al., 2021). The tested cultivars did not show significant differences in SPAD value; hence, nitrogen application enhanced flag leaf chlorophyll content in the study. The nitrogen x cultivar interaction for flag leaf SPAD value showed significant (p<0.01) differences. The highest SPAD flag leaf value was determined in the Alatay cultivar with 100 and 150 kg ha^{-1} also besides this cultivar highest SPAD value was obtained from Poyraz under 100 kg ha^{-1} nitrogen applications. Genotypes with slow senescence and having the lowest chlorophyll loss were able to retain stay green properties and this situation makes a positive contribution to main yield characteristics at the beginning of anthesis to early milky development stages (Bahar, 2015). Water deficiency and nitrogen availability in post-anthesis development periods are the main limiting factors affecting durum wheat productivity (Lopes and Araus, 2006). Physiological traits affecting genetic potential include flag leaf photosynthesis rate, post-anthesis N remobilization, and leaf senescence kinetics. In wheat 35-42% of the above-ground N accumulated at anthesis in leaf lamina and higher flag leaf N remobilization rate and stem re-maintaining to leaf N were associated with delayed leaf senescence (Nehe et al. 2020). In the study, inadequate nitrogen supply caused a decline in flag leaf chlorophyll content and so this finding highlights just how important sufficient nitrogen storage of durum wheat is important, especially in the post-anthesis period which determines yield potential (Figure 2.).

Durum wheat quality properties mainly depend on seed companies, grain dealers, farmers, milling industry, pasta industry and consumers so it has not been possible to propose simple and exhaustive information. The quality of end-use products is related to consumer awareness and quality of durum wheat, which is mainly determined by the genotype, but also by environment (weather conditions and nutrition status) and crop management (Troccoli et al. 2000). Protein content plays an important role in quality for durum products. High level of protein content is required for good-looking durum products. Grain protein content higher than 13% is suitable for making good quality pasta and also enhances the tolerance to overcooking (Saini et al. 2022). Protein content showed statistically significant (0.01)

differences depending on the nitrogen doses. The nitrogen application dose with the highest protein content (16.6%) was 150 kg ha^{-1}, while the lowest value (13.1 %) was determined for 50 kg ha^{-1}. Nitrogen application with the highest level (150 kg ha^{-1}) caused an increase in protein content, especially in Alatay and Ç 1252 cultivars also in addition to these values Şölen cultivar with 100 kg ha^{-1} nitrogen dose also had the highest protein content (Figure 2.). The average grain protein content values ranged in a wide spectrum between 11.2-18.6% depending on nitrogen applications and cultivars. The obtained protein results are mainly consistent with previous studies (Chen et al. 2022) however it is found slightly higher values (16-18%) for protein content with respect to those reported by (Akgün and Ulupınar (2019); Doğan and Cetiz (2015); Değirmenci (2017). High protein content is related to grain hardness and pasta raw material producers favor and also enables it not to crumble and firm after cooking pasta by reason of high protein content semolina (Makowska et al. 2008).

Ash content is regarded as one of the main quality characteristics of durum wheat grains as it has an influence on pasta color. Grains with high ash content cause to faint color of semolina and may be due to high extraction rates. The obtained pasta tends to have a brown color and is generally preferred to have premium-grade semolina lower than 0.9% ash content (El-Khayat et al. 2006). The ash contents of different durum wheat cultivars showed significant differences (p<0.01) among them and varied from 0.63% (Alatay) to 0.79% (Poyraz). Nitrogen fertilization doses also caused significant differences in the ash content and it was revealed that the amount of ash content in the grain tended to decrease with increasing nitrogen fertilizer applications (Table 2). In contrast to earlier findings (Marino et al. 2009) reported as N application had no clear effect on ash content in durum wheat, while higher values were obtained in ash content with increasing N rates. The highest ash content was determined in the Şölen cultivar with non-applicated nitrogen fertilization (1.01%) whereas the lowest was determined in the Alatay cultivar with 100 kg ha^{-1} nitrogen fertilization dose (Table 2 and Figure 1). The ash values for whole grain are at low levels compared to those typically observed in previous studies (El-Khayat et al. 2006; Yıldırım and Atasoy, 2020; Akgün et al. 2021).

Wheat germ is generally removed during milling process, although its rich in chemical compounds for health. It contains high amount of proteins, carbohydrates, as well as water, and lipids. Moreover, whole wheat which contains germ part has health-promoting compounds such as flavonoids, polyphenols, tocopherols, and tocotrienol i.e. vitamin E (Naureen et al. 2022). Nitrogen fertilization applications caused statistically significant differences (p<0.05) in terms of the lipid content of durum wheat (Table 2.). The highest lipid content was determined in the 50 kg ha^{-1} (2.57 %)

whereas the lowest was in the 0 (2.20 %), 100 (2.16%), and 150 kg ha^{-1} (1.95%) nitrogen doses. Nitrogen x cultivar interaction showed statistically significant differences (p<0.01) and Ç 1252 cultivar had the highest value with non-applied nitrogen fertilization (0 kg ha^{-1}). In general, lipid content had no clear response to nitrogen fertilization with regard to applied doses to cultivars (Table 2 and Figure 1). The lipid content values found in the study are in line with those reported by Ounane et al. (2006) and Değirmenci (2017) but higher lipid values reported by Narducci et al. (2019) compared to obtained lipid content results.

Table 2. The effects on nitrogen and cultivar on grain quality parameters

Ash %		Ç 1252	Poyraz	Şölen	Alatay	Mean nitrogen
	0 kg ha^{-1}	0.90 abc	0.97 ab	1.01 a	0.85 a-d	0.93 A
	50 kg ha^{-1}	0.51 fg	0.88 abc	0.84 a-d	0.59 efg	0.70 B
	100 kg ha^{-1}	0.54 fg	0.79 b-e	0.57 fg	0.40 g	0.67 BC
	150 kg ha^{-1}	0.78 b-e	0.53 fg	0.70 c-f	0.67 def	0.57 C
	Mean cultivar	0.68 BC	0.79 A	0.78 AB	0.63 C	
	$Lsd_{Nitrogen}$: 0.08; $Lsd_{Cultivar}$: 0.11; $Lsd_{Interaction}$: 0.20; F $value_{Nitrogen}$: 15.8**; F $value_{Cultivar}$: 4.2**; $F_{Interaction}$: 5.8**					
		Ç 1252	Poyraz	Şölen	Alatay	Mean nitrogen
Lipid %	0 kg ha^{-1}	2.92 a	2.09 b-e	1.67 cde	2.15 a-e	2.20 B
	50 kg ha^{-1}	2.39 abc	2.75 ab	2.78 ab	2.37 a-d	2.57 A
	100 kg ha^{-1}	2.06 b-e	2.25 a-e	1.55 e	2.77 ab	2.16 B
	150 kg ha^{-1}	1.57 e	2.49 ab	2.16 a-e	1.60 de	1.95 B
	Mean cultivar	2.23	2.39	2.04	2.22	
	$Lsd_{Nitrogen}$: 0.35; $Lsd_{Interaction}$: 0.78; F $value_{Nitrogen}$: 3.2*; F $value_{Interaction}$: 2.6**					
		Ç 1252	Poyraz	Şölen	Alatay	Mean nitrogen
Fiber %	0 kg ha^{-1}	2.79	2.84	2.67	2.86	2.79 B
	50 kg ha^{-1}	3.14	2.96	2.80	2.74	2.91 B
	100 kg ha^{-1}	3.00	3.35	3.93	3.68	3.49 A
	150 kg ha^{-1}	3.45	3.07	3.00	3.51	3.25 A
	Mean cultivar	3.09	3.05	3.10	3.20	
	$Lsd_{Nitrogen}$: 0.31; F $value_{Nitrogen}$: 321.2**					
		Ç 1252	Poyraz	Şölen	Alatay	Mean nitrogen
Protein %	0 kg ha^{-1}	12.7 ef	15.9 b	15.2 bcd	14.6 b-e	14.6 B
	50 kg ha^{-1}	13.6 cde	14.1 b-e	11.2 f	13.3 def	13.1 C
	100 kg ha^{-1}	15.7 bc	14.0 b-e	18.4 a	16.1 b	16.0 AB
	150 kg ha^{-1}	18.4 a	14.8 b-e	14.5 b-e	18.6 a	16.6 A
	Mean cultivar	15.1	14.7	14.8	15.7	
	$Lsd_{Nitrogen}$: 1.5; $Lsd_{Interaction}$: 2.2; F $value_{Nitrogen}$: 9.2**; F $value_{Interaction}$: 2.9**					
		Ç 1252	Poyraz	Şölen	Alatay	Mean nitrogen
Starch %	0 kg ha^{-1}	60.0 ef	61.8 c-f	61.8 c-f	63.7 a-d	61.8 AB
	50 kg ha^{-1}	60.9 def	62.3 cde	63.4 bcd	66.5 a	63.3 A
	100 kg ha^{-1}	61.7 def	65.3 ab	59.5 ef	58.9 f	61.4 B
	150 kg ha^{-1}	55.6 g	64.8 abc	62.1 cde	55.0 g	59.4 C
	Mean cultivar	59.6 C	63.5 A	61.7 B	61.0 BC	
	$Lsd_{Nitrogen}$: 1.7; $Lsd_{Cultivar}$: 1.5; $Lsd_{Interaction}$: 3.0; F $value_{Nitrogen}$: 9.5**; F $value_{Cultivar}$: 9.9**; F $value_{Interaction}$: 8.2**					

Bran is a key factor in identifying whole-grain benefits and contains various amounts of bioactive compounds. Wheat bran is clearly the highest

in dietary fiber and health-promoting components compared to other parts of grain. Dietary fiber includes cellulose, lignin, hemicellulose, and other polysaccharides associated with plant and defined as edible parts of plants that are resistant to digestion also whole grain products (contains bran) has more health benefits compared to fiber-free products (Esposito et al. 2005). In terms of fiber content of grains, no statistically significant differences were observed between cultivars and the combined effect of nitrogen and cultivars (interaction) but fiber content responded significantly (p<0.01) to nitrogen fertilization doses (Table 2). The fiber content of grain showed an increasing trend to higher nitrogen fertilization doses, the highest fiber content was obtained from 100 and 150 kg ha^{-1} applications while the lowest values were recorded in 0 and 50 kg ha^{-1} nitrogen fertilization doses. It was found that nitrogen fertilization affected fiber content of grain and resulted in higher values whereas Khalil et al. (1987) and Jańczak-Pieniążek et al. (2020) found that fiber content did not change significantly.

Starch is a major contributor of yield, accounting for 65-75 % of the grain dry weight and up to 80 % of the endosperm dry weight. Reductions and shortened starch accumulation during grain filling period account for significant losses in grain yield and weight (Dupont and Altenbach, 2003). Nitrogen fertilization doses caused statistically significant changes in the starch content of durum wheat grain. Grain starch content declined significantly under the high nitrogen treatments (100 and 150 kg ha^{-1}) compared to non-applied and 50 kg ha^{-1} nitrogen fertilization doses. These results correlate favorably with Ben-Mariem et al.'s (2020) findings that explained starch concentration decreased with increasing nitrogen availability. The researchers underlined the situation that under the high nitrogen treatment, grain carbohydrates tend to be stored as glucose, sucrose, and maltose not as starch resulting in decelerated starch synthesis. The highest nitrogen application caused a decrease (approx. -4 %) in the amount of starch compared to non-applied (0 kg ha^{-1}) nitrogen fertilization. Genotypic variability also influenced starch content of grain and the highest starch value was determined in the Poyraz (63.5 %) whereas the lowest value was determined in Ç 1252 (59.6 %) cultivar. The starch content of cultivars varied between 55.0 % and 66.5 % under different nitrogen fertilization doses. The highest starch content was found in the Alatay cultivar with 50 kg ha^{-1} nitrogen dose whereas the lowest value was obtained from Alatay and Ç 1252 cultivars with the highest nitrogen level (150 kg ha^{-1}). Poyraz drew attention by observing that it responded positively to increasing nitrogen doses by taking higher values compared to other cultivars.

HunterLab flour color values (L*, a*, b*)

The color of the grain and durum products arises from phenotypic variations in the pigments present in the grain. The grain color depends on genetic factors, growing conditions, and production processes. In particular, in terms

of genetic control responsible genes in pigment accumulation code both for enzymes involved in pigment biosynthesis and degradation and also for proteins as regulatory roles (Ficco et al. 2014). In light of this information, genetic factor was only found statistically significant in L* (brightness) value except for a* (redness) and b* (yellowness) values. Contrary to the genetic factor, nitrogen fertilization caused a significant change in the all color values of durum wheat. The most striking result is higher nitrogen fertilization doses (100 and 150 kg ha^{-1}) increased yellow and red pigments in grain while the brightness of grain lowered with higher nitrogen applications. Higher nitrogen application doses are also accompanied by the increase in the protein content of grain together with red and yellow-colored pigments (Table 1, Table 2). The results point to the likelihood in the previous study explained that durum wheat contains high protein content with yellow-colored pigment which is the most suitable wheat quality trait that makes it the perfect choice to make pasta, semolina, and spaghetti (Saini et al. 2022). Taken together, all these findings suggest that higher nitrogen application would seem to increase the quality properties of durum wheat in product processing.

For the data of flour brightness color (L*), shown in Table 3, the highest value was observed in 50 kg ha^{-1} (85.3%) while the lowest values were obtained from 100 kg ha^{-1} (83.5%) and 150 kg ha^{-1} (83.2%) applications. The result findings appear to be well supported by Yıldırım and Atasoy (2020) obtained L* values between 82.92% and 84.86% also researchers explained that flour samples that has L* values exceed 85% for all of them due to high starch content which in this study can be underlined 50 kg ha^{-1} had the highest starch content and brightness value (Table 2 and 3). The average brightness values depending on the cultivars varied between 83.6% and 84.9% and Şölen had the highest L* value among other cultivars. Nitrogen x cultivar interaction was found statistically significant ($p<0.01$) in the L* value and ranged between 82.4% (Ç 1252, 150 kg ha^{-1}) and 87.9% (Şölen, 50 kg ha^{-1}). The other HunterLab color value a* (redness) changed significantly ($p<0.01$) under different nitrogen fertilizer doses. Higher nitrogen fertilizer application increased the redness color in durum wheat and the highest value was achieved in 150 kg ha^{-1} (2.79%) and 100 kg ha^{-1} (2.65%) while the lowest values (2.42 and 2.36%) were obtained from 0 and 50 kg ha^{-1} doses. As seen in Table 3, there are statistically significant differences ($p<0.01$) of nitrogen x cultivar interaction and the values ranged from 2.08 to 3.01% observed in Şölen with 50 kg ha^{-1} and Ç 1252 cultivar with 150 kg ha^{-1} for the redness pigment of flour, respectively.

The b* yellowness color of flour value that one of the most important quality characteristics for pasta, bulgur, and other products was only affected by nitrogen fertilization doses, and higher nitrogen fertilization doses triggered to increase b* value. The highest b* value was found in 100 kg ha^{-1} (19.6%) and 150 kg ha^{-1} (20.0%) while the lowest was observed in 0 kg ha^{-1} (17.8 %) and 50 kg ha^{-1} (18.2%) nitrogen doses.

Table 3. Average values of durum wheat flour color (L, a*, b*)*

		Ç 1252	Poyraz	Şölen	Alatay	Mean nitrogen
L* Brightness	0 kg ha^{-1}	84.3 bcd	84.2 b-f	84.8 b	84.7 bc	84.5 B
	50 kg ha^{-1}	84.2 b-e	84.9 b	87.9 a	84.4 bcd	85.3 A
	100 kg ha^{-1}	83.5 c-g	83.6 b-g	83.0 fg	84.1b-f	83.5 C
	150 kg ha^{-1}	82.4 g	83.4 d-g	84.0 b-f	83.0 efg	83.2 C
	Mean cultivar	83.6 B	84.0 B	84.9 A	84.0 B	
	$Lsd_{Nitrogen}$: 0.5; $Lsd_{Cultivar}$: 0.6; $Lsd_{Interaction}$: 1.2; F value $_{Nitrogen}$: 17.7**; F value $_{Cultivar}$: 5.6**; F value $_{Interaction}$: 7.2**					
		Ç 1252	Poyraz	Şölen	Alatay	Mean nitrogen
a* Redness	0 kg ha^{-1}	2.34 ef	2.59 b-e	2.32 ef	2.43 e	2.42 B
	50 kg ha^{-1}	2.54 cde	2.34 ef	2.08 f	2.48 de	2.36 B
	100 kg ha^{-1}	2.61b-e	2.52 de	2.88 ab	2.62 b-e	2.65 A
	150 kg ha^{-1}	3.01 a	2.85 abc	2.55 b-e	2.77 a-d	2.79 A
	Mean cultivar	2.62	2.57	2.46	2.58	
	$Lsd_{Nitrogen}$: 0.2; $Lsd_{Interaction}$: 0.3; F value $_{Nitrogen}$: 13.5**; F value $_{Interaction}$: 4.0**					
		Ç 1252	Poyraz	Şölen	Alatay	Mean nitrogen
b* Yellowness	0 kg ha^{-1}	16.4	18.0	18.6	18.3	17.8 B
	50 kg ha^{-1}	18.6	19.0	17.0	18.3	18.2 B
	100 kg ha^{-1}	19.7	20.7	19.4	18.7	19.6 A
	150 kg ha^{-1}	20.6	20.5	19.4	19.7	20.0 A
	Mean cultivar	18.8	19.5	18.8	18.6	
	$Lsd_{Nitrogen}$: 1.0; F value$_{Nitrogen}$: 9.2**					

Antioxidant activity and total phenol content

Durum wheat grain quality is affected by many factors and it is necessary to understand which factors modify the chemical composition and health aspects of grain that limit producing healthy foods. As it mentioned above nitrogen fertilization amounts caused significant changes in yield, quality, and pigment properties of durum grain. In this part of the chapter, it is wondered and evaluated how nitrogen fertilization may alter antioxidant activity and phenol content of durum wheat. The results of ANOVA related to four cultivars, grown over four nitrogen dose levels are given in Table 4. The analysis shows that cultivar and its interaction with nitrogen significantly affect total phenolic content of durum wheat. Nevertheless, total phenol content of durum wheat was not affected by applied nitrogen fertilizer doses. Among the cultivars apart from Poyraz cultivar (196.8 µg GAE/g), Şölen (213.2 µg GAE/g), Alatay (209.4 µg GAE/g) and Ç 1252 (209.1 µg GAE/g) cultivars had the highest phenol content values. The mean value of cultivar and nitrogen application interaction ranged from 180.7 to 241.1 µg GAE/g and the values achieved in Poyraz cultivar with 100 kg ha^{-1} and Alatay cultivar with 100 kg ha^{-1}, respectively. And also the highest and lowest values were obtained from the same nitrogen dose (100 kg ha^{-1}) so it may be concluded that the response of cultivars to fertilization is important for changes in phenol content of grain but further analysis is needed to explain this situation. The results of this study confirm by the previous researchers found total phenolic concentration was

not affected by N treatments in wheat grain (Stumpf et al. 2019). When the obtained results were compared to previous studies, it was observed that lower values were found in total phenol content in the study (Žilić et al. 2011; Žilić et al. 2013; Zrcková et al. 2018).

The effect of N fertilization was significant (p<0.01) for DPPH radical scavenging activity of durum wheat grain, indicating a slight decrease in antioxidant activity as N rates increased. Compared to the non-applied fertilizer rate (0 kg ha^{-1}), the highest applied fertilizer dose (150 kg ha^{-1}) caused an approx. 14 % decrease in antioxidant activity. In regard to antioxidant activity the highest (49.8 %) and lowest (32.1 %) mean value of nitrogen x cultivar interaction was achieved in the same cultivar (Ç 1252) with non-applied (0 kg ha^{-1}) and the highest nitrogen rate (150 kg ha^{-1}), respectively. Poyraz cultivar had also the lowest value (33.2 %) with 150 kg ha^{-1} nitrogen fertilizer dose. Ç 1252 responded with a high decrease in antioxidant activity compared to other cultivars while Alatay cultivar showed the opposite effect.

Table 4. Average values for total phenol content (µg GAE/g) and antioxidant activity (% inhibiton) mean values

		Ç 1252	Poyraz	Şölen	Alatay	Mean nitrogen
Total phenol content (µg/g)	0 kg ha^{-1}	209.9 bc	192.2 cd	224.1 ab	208.4 bc	208.6
	50 kg ha^{-1}	213.7 bc	203.3 bcd	206.8 bc	181.5 d	201.3
	100 kg ha^{-1}	200.2 cd	180.7 d	210.8 bc	241.1 a	208.2
	150 kg ha^{-1}	212.7 bc	210.9 bc	211.1 bc	206.5 bc	210.3
	Mean cultivar	209.1 A	196.8 B	213.2 A	209.4 A	
	$Lsd_{Cultivar}$: 11.4; $Lsd_{Interaction}$: 23.7; F value $_{Cultivar}$: 3.3**; F value $_{Interaction}$: 2.9**					
		Ç 1252	Poyraz	Şölen	Alatay	Mean nitrogen
Antioxidant activity (%)	0 kg ha^{-1}	49.8 a	43.8 b	40.6 cde	36.8 f	42.8 A
	50 kg ha^{-1}	38.6 ef	41.7 bcd	41.9 bcd	40.4 cde	40.7 B
	100 kg ha^{-1}	42.1 bcd	42.9 bc	39.6 def	42.5 bcd	41.8 AB
	150 kg ha^{-1}	32.1 g	33.2 g	41.7 bcd	42.9 bc	37.5 C
	Mean cultivar	40.7	40.4	40.9	40.6	
	$Lsd_{Nitrogen}$: 1.6; $Lsd_{Interaction}$: 3.0; F value $_{Nitrogen}$: 17.9**; F value $_{Interaction}$: 14.3**					

The observed antioxidant activity values are mainly in accordance with the previous study conducted by Ben-Mariem et al. (2020). In general, cultivars responded differently with changing antioxidant activity to nitrogen fertilization but there were no statistically significant differences in cultivars for antioxidant activity. The findings of this study differ from Ben-Mariem et al. (2020) mentioned that changes in total polyphenols concentration significantly increased in (+25.21 %) the highest polyphenol accumulation being observed in low-yielding genotypes. The comparison between the high and low N treatments gave similar antiradical scavenging activity and no significant changes were observed according to antioxidant activity under different nitrogen treatments. Although all together researchers concluded that there was a genotype x treatment interaction at low N, the low-yielding genotypes had more antirad-

ical activity, but with high N, it was the other way around. In this study findings also may correlate when it is compared grain yield and antioxidant activity explained in mean values with heat map for observed characteristics together with uncertain results (because no significant correlation was found between two parameters) there is a trend in increasing grain yield caused lower antioxidant activity except Alatay 150 kg ha^{-1} application.

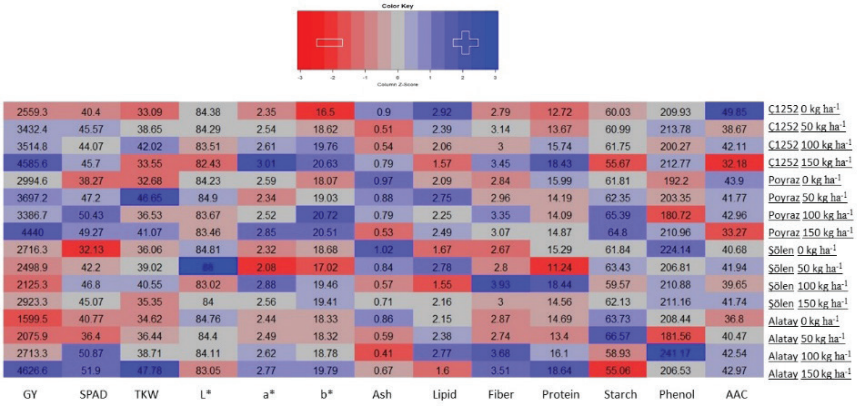

Figure 1. Mean values with heat map for observed characteristics

Correlation results of examined characteristics

Few studies have been conducted to reveal the effect of N fertilization on the antioxidant properties of durum wheat in Turkey, and its correlation with yield and quality. Examining in Figure 2, Pearson correlation results were examined with significance levels (p<0.05, p<0.01, p<0.001) of the evaluated parameters. One of the most significant practical outputs of this study is that SPAD readings in flag leaf during the grain filling period resulted in significant positive linear relationships between SPAD chlorophyll content and yield (r=0.52***), TGW (r=0.50***), quality [protein (r=0.30*); fiber (r=0.59***)] and flour color [a*(r=0.42**); b*(r=0.43**)] parameters. The increased availability of nitrogen ensured a higher concentration of chlorophyll in foliar tissues and a close relationship between SPAD readings and leaf chlorophyll concentration was observed by Fiorentini et al. (2019). Higher nitrogen contribution to durum wheat responded to higher SPAD readings that give information about the chlorophyll content of flag leaf (Table 1). There is a growing interest in using chlorophyll meter in durum wheat breeding might give important information to breeders and agronomists to obtain information more effectively and quickly about yield and quality characteristics in field conditions. These results were supported by the findings of Rharrabti et al. (2001); Rafiqul Islam et al. (2014); Kızılgeçi et al. (2019). Grain yield resulted in a positive and significant relationship with TGW (r=0.43**) and increasing durum wheat grain weight caused higher yield values. On the other hand, it was observed that a significant

linear relationship between yield and flour color [a* redness (r= 0.40**); b* yellowness (r=0.47***)] while negative and significant relationship was observed in brightness L* value (r=-0.39**). The obtained significant correlation result with grain yield and durum wheat color pigments is attractive to have both high yield and quality products but this situation must be supported by other researchers and future studies.

One of the most important quality and breeding goal in durum wheat is the potential of producing semolina and pasta products with a bright yellow color and release of cultivars with high pigment levels. Protein content is crucial for durum wheat products and pasta-cooking quality so obtaining higher protein content values is preferred in durum wheat production (Sarkar and Fu, 2022). One of the most important result in this study is a significant positive correlation observed between protein content and flour yellowness (r=0.46**) so higher protein content increased flour yellowness of durum wheat. All together flour color and grain protein content are determining factors for the end-use quality of durum products so correlation results showed that with an increase in protein content contributed to obtain yellow-amber color of durum wheat grain (Saini et al. 2022). The brightness color of flour (L*) had negative significant correlation results with some yield and quality characteristics. It was determined that negative correlation results were observed in grain yield (r=-0.39**), SPAD chlorophyll content (r=-0.35*), protein content (r=-0.64***), and fiber content (r=-0.44**). On the other hand brightness color of flour showed statistically positive significant correlation results with starch content (r=0.31*), lipid content (r=0.41**) and ash content (r=0.34*). HunterLab color values are correlated significantly with each other. A linear significant relationship (r=0.64**) was observed between a* redness and b* yellowness color. Millers prefer to use high-pigment durum wheat for blending to improve the color of semolina and pasta products. It is important to achieve both higher redness and yellowness values and there is a slight elevation of pasta redness with the increase of total yellow pigments in durum wheat (Sarkar and Fu, 2022).

Significant correlation results were also observed in each other of quality characteristics. Protein and starch contents were found to be inversely correlated (r=-0.61***) which is in line with previous studies (El-Khayat et al. 2006; Erekul and Köhn 2006). There is an unfavorable result between protein and lipid content with a negative correlation (r=-0.70***) because lipids are the key biochemical compounds of wheat and have an important impact on cooking quality (Ounane et al 2006). Fiber content outer layer of the grain offers healthy benefits and utilization in the diet can help protect from heart disease, insulin secretion, and cancer (Nirmala Parasadi and Joye, 2022). Although fiber is mainly contributed to durum wheat products and its proportion is important, significant negative correlation results were obtained as a result of the study. Fiber content was negatively correlated with protein (r=-0.58***), starch (r=-0.40**), and ash

(*r*=-0.49***) content and this situation may be related to their inversely effects each other and the pathway of compounds in grain development.

Total phenol content and antioxidant activity had weak correlation results compared to other characteristics. The most attractive correlation result was observed between fiber and phenol content of durum wheat grain. As seen in Figure 2. significant and positive relationship between total phenol content and fiber content (*r*=0.31*) was observed. The Bran part of the grain has the highest dietary fiber with health-promoting components and its fundamental contribution is explained by the fact that phenol compounds are associated with the outer layers (Esposito et al. 2005). Significant negative correlation results were also reported in Figure 2. between antioxidant activity and flour color parameters [a* redness (*r*=-0.38**) and b* yellowness (*r*=-0.37*)]. The obtained correlation results were not matched with some carotenoids (yellow pigment) that have provitamin A activity provides protection from chronic diseases and all carotenoids show antioxidant capacity which reduces the risk of the diseases (Ficco et al. 2014).

In this part of the study relationship between yield, quality, and antioxidant potential of durum wheat is generally considered but it is important to express these correlation results must be evaluated with multi-year experiments so uncertain results can be clarified.

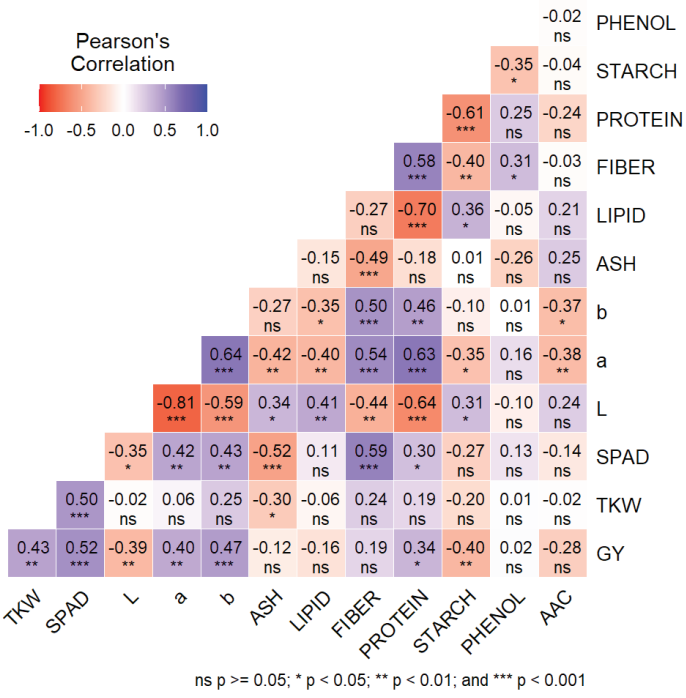

Figure 2. Pearson correlation results with correlogram

CONCLUSION

The results of yield, grain weight, and SPAD chlorophyll value highlight that higher N supply promoted to get higher values. As it mentioned correlation results above flag leaf SPAD readings may give important information to reach the favourite yield and quality results for durum wheat by using SPAD meter as an indirect selection criterion in durum wheat studies. It can be concluded that the maintenance nitrogen status of flag leaf during the grain filling period ensured to the achievement of higher yield and protein content values by higher chlorophyll content. End-use quality and nutritional value of durum wheat were also evaluated and the application of nitrogen with higher doses has resulted with increasing protein content which is the main component of durum wheat quality. Additionally, HunterLab flour color values (a* and b*) increased with higher N supply and the most important quality characteristic for pasta and other durum products is yellowness pigment (b*) responded positively together with protein content. Research has also been focused to investigate the relationship between N and antioxidant response of durum wheat. N fertilization had significant effects on the anti-radical activity when considering all nitrogen doses; slightly decreased results were achieved with N supply and also increasing trend was observed in higher grain yield caused lower antioxidant activity.

These findings might be useful for understanding nitrogen fertilization has a greater impact on yield, quality, pigment, and health benefits of durum wheat also in relation to the choice of more suitable nitrogen application place a premium situation on to obtain high quality and healthy durum products. Finally, the present results of this chapter demonstrated how durum wheat quality and antioxidant properties responded to nitrogen fertilization and reported relationships between evaluated parameters. Nitrogen fertilization found to be an important factor to achieve healthy, nutritionally superior and high quality durum wheat produts but the obtained results must be evaluated by the future studies with multiple years and together with environmental aspects.

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CHAPTER 3

CLIMATE CHANGE AND BEES

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INTRODUCTION:

Climate change is one of the most contentious worldwide topics of the twenty-first century. The Earth's average surface temperature increased by around 0.6°C over the previous century (NAST 2000), and it is expected to rise to 4°C by 2100. (Thuiller, 2007). Several changes in our climate may occur as a result of this increase in temperature, such as shifting rainfall distribution and the frequency of violent weather events (Weyant and Yanigisawa, 1998). Meanwhile, it is predicted that global warming would raise sea levels by a few feet by the end of the century, and hurricanes, heatwaves would become more often than they are now (Barth and Titus, 1984; Adediran et al., 2023).

According to several studies, global warming is both a concern for available ecosystems (Hughes, 2000; Zscheischler et al. 2018; Gusev 2022) and one of the biggest threats to the world's biodiversity in the long term future (Hughes, 2000; Root et al., 2003; Harley et al., 2006; Prakash, 2021).When biodiversity is destroyed, food, fuel, construction materials, medicinal, and genetic resources will decline. This alteration might also affect the population of other species. In light of the fact that biodiversity is nature's insurance, it is vital to design measures to conserve it.

The evidence is mounting that human activities (Brazil, INPE, 2002; Stern, 2006; Lonngren and Bai, 2008) are to blame for the majority of the global warming that has happened in the previous 50 years (Brazil, INPE 2002, Stern 2006, Lonngren and Bai, 2008; IPCC 2007; He and Silliman 2019). The combustion of fossil fuels and deforestation are two examples of anthropogenic emissions that contribute to climate change. Carbon dioxide (CO_2) and other 'greenhouse' gases are emitted by both of them (Brazil, INPE 2002, Stern 2006, Lonngren and Bai, 2008; Fawzy et al. 2020) Water vapor, ozone, and methane, in addition to CO_2, are other greenhouse gases that impact Earth's temperature although making up less than 0.1 percent of the atmosphere (Srinivasan, 2008). Despite the fact that human emissions from industry, deforestation, and agriculture have been left over in the most appropriate way since the beginning of the Industrial Revolution. However, since the 1950s, worldwide emissions have increased at an alarming rate (IPCC 2007). From 1970 to 2004, worldwide emissions climbed by 70%, and CO_2 emissions were nearly twice as high as they were in the1970s (IPCC, 2007). In order to prevent climate change, global greenhouse gas emissions must be significantly decreased.

Despite the fact that global climate change is thought to have a wide range of consequences, one of the most significant is expected to be on agriculture (Nordhaus, 1991; Pearce, 1996; Cline, 2007; Arora, 2019). Agriculture, which provides a wide range of ecosystem services, is a ma-

jor economic, social, and cultural activity. Climate change has a significant impact on many different aspects of agriculture in high-sensitivity locations. The El Nino Southern Oscillation phenomenon, which causes a cycle of droughts and floods events, is responsible for 15 to 35 percent of worldwide performance variation in wheat, oilseeds, and coarse grains (Ferris, 1999), making them too susceptible to global temperature change. An rise in the average seasonal temperature may shorten the growth season of several crops, hence reducing their output. The sensitivity of food production systems to climatic changes, such as fluctuations in temperature and precipitation, may lead to outbreaks of pests and diseases, consequently lowering the harvest and threatening the country's food security.

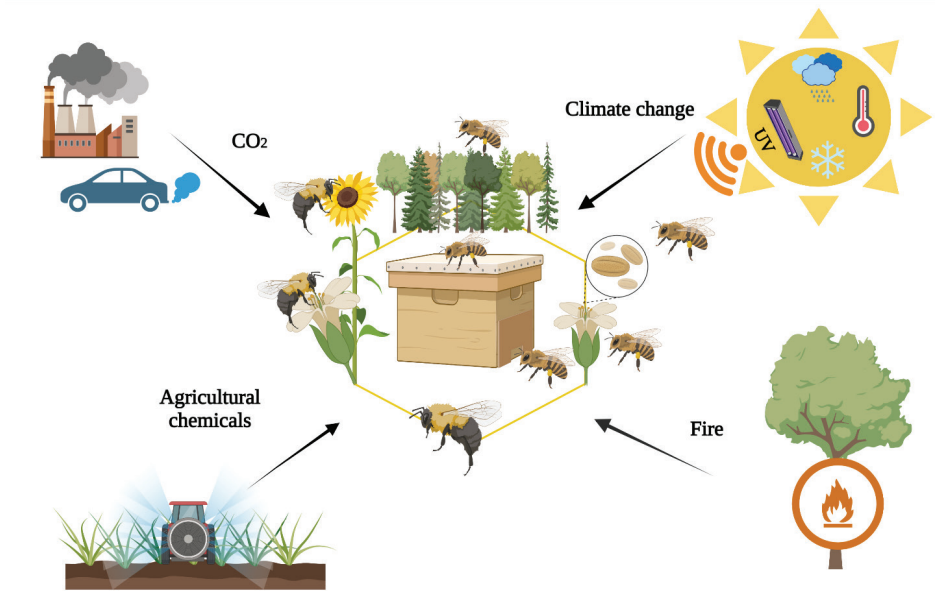

Figure 1.*Negative factors of bees (by Biorender.com)*

Numerous scientific research demonstrate that climate change impacts a wide range of organisms (Hughes, 2000; Walther et al., 2001; Cavicchioli et al., 2019). This study aims to demonstrate how climate change influences the population dynamics, physiology, and behavior of bees, as well as plant-pollinator interactions.

1. Effects on Bees

1.1. Population Dynamics

Insects, particularly those of the order Hymenoptera, play an equally important role in plant pollination as wind does. Among the 20,000 species of bees (Apoidea), only a small number of social and solitary species have been managed for commercial crop pollination. Most bee species have solitary lives, with females building nests and rearing their own offspring. Solitary bees do not have a queen or worker caste, instead consisting of a large number of females who may or may not be related, with a low reproductive skew and no fighting among nest members (Danforth et al. 2019). Solitary bees are unable to manage the temperature in their nest and brooding space. The reproductive castes of queens and workers describe social societies with a high degree of reproductive skew.

Queens lay all or most of the eggs and are extremely aggressive, especially toward workers (Orlova et al., 2020) . Second, communal societies comprise adults from the same generation, whereas social societies comprise workers descended from a queen (Richard et al., 2003). They have the ability to manage the temperature in their nest and brood habitat.

As with Apis or honey bees, comparative studies of heat generation and management by foraging bees concentrate on variables including mass-specific metabolic rate, flying speeds, and wing area to body mass ratio (Dyer and Seeley, 1987). Dyer and Seeley (1987) found that several of these parameters change disproportionally across four species of the genus Apis and demonstrated that there are broad implications for many behavioral traits. There might potentially be selection for characteristics that facilitate environmental temperature adaptability.

Africanized honey bees can survive in the wild. They have adapted to tropical conditions. If global warming continues, it may spread over the world, including North America. These provide issues for the beekeeping sector. In addition, they may have to contend with other pollinators that will be similarly impacted by climate change.

1.2. Temperature changing effects on bee brood and foraging behavior

Climate change has a greater impact on brood rearing in solitary bees than in social bees. None of the solitary or semi-social bees manage the temperature of their nests. In contrast, Ostwald et al., (2022) discovered that carpenter bees, who are asocial yet live in colonies, preserve body heat and bulk better than solitary bees over the winter. Significant temperature changes would thus be detrimental to solitary bees' nest brood, but prim-

itively social bees, such as bumblebees and honey bees, would carefully manage nest and brood area temperatures.

Honey bees can maintain an incubation temperature of 30-35 degrees Celsius above the ambient temperature range of -40 to -40 degrees Celsius. Honey bee workers may increase their body temperature without flapping their wings by engaging their flying muscles in the thorax. The temperature differences between the thorax and abdomen of the workers in the cluster's outermost layer and those of their neighbors are negligible. When their thorax temperature spikes from 10°C, 23-27°C to 33-37°C within minutes, however, worker bees in the outside shell of the cluster periodically travel into the center of the cluster. They are able to remain in the heart of the cluster for up to 12 hours, raise their body temperature when they begin to descend, and finally rejoin the worker bees in the outer shell. Thus, the cluster may be seen as a dynamic system in which heat is created metabolically in the loose center cluster and retained by the tighter insulating outer shell, with individuals performing both heating and insulation roles at various periods. Honey bees not only raise the nest's temperature during low air temperatures, but also protect it from overheating at high ambient temperatures (Heinrich, 1985). In response to the high warmth in the hive, the honeycomb-clustering bees scatter. If the nest cannot be properly cooled by distributing the bees around the honeycombs, the worker bees may gather outside the hive. Bees leaving the colony are expected to lower their body temperature while also making space inside the hive for air circulation and cooling. At high temperatures, the growth of winter clusters has the same effect. If heat stress persists in the hive, the bees will start distributing and transporting water that evaporates and cools the hive to the honey comb cells. Forager worker bees switch from nectar collection to nectar or water collection as hive temperatures rise and the need for water for evaporative cooling rises (Winston, 1987).

Jones et al (2007). This study discovered that genetic variation across paternal lineages is an important factor in the job of *Apis florea* fanning employees. Even though bees have a thermoregulation mechanism, their foraging performance will vary if the ambient temperature continues to rise as a result of climate change. Due to the excessive temperature, they will only go short distances. This will negatively affect the population dynamics of the colony. Because foragers will be unable to carry as much food back to their colonies, there may be a reduction in brood production. Food shortages may diminish the size of bees' bodies and colonies, as well as damage their immune systems.

2. Physiology and Behavior

The cost of bee body temperature regulation is influenced by climatic conditions. Bees have adapted their anatomy to transmit and retain heat in their flying muscles, or to shift heat away from the thorax and into the abdominal cavity or head, where it is disseminated (May and Casey, 1983). In several genera, such as Bombus, Apis, Melipona, Euglossa, and Eulaema, the aortic patch has developed into a series of folds around the thoracic base (May and Casey, 1983). This arrangement allows at least some Bombinae and potentially bigger meliponins with a comparable thoracic aorta to redirect warm blood from the thorax to the abdomen, where heat is dissipated when cooler hemolymph from the abdomen is pumped into the thorax. During the pre-flight warming up of the muscles, the contraction of the flight muscles prevents the pumping of the abdominal muscles and, therefore, the increased blood flow from the heart to the rear of the belly. Thus, there is no heat transmission to the abdomen. However, the curved form of the honeybee dorsal vein limits the effective transmission of heat to the abdomen; instead, the majority of this heat is held in the rib cage, enabling the abdomen's hemolymph to warm. The honey bee directs excess heat to its head, which cools by pulling nectar from its stomach and evaporating it as it travels (Heinrich, 1980).

Flight needs a minimum thoracic temperature of 25-30 0C. (May and Casey, 1983; Heinrich and Buchmann, 1986). Honey bee thoracic temperatures are normally 10 - 15 °C over ambient, but may reach 23 °C above ambient after returning from foraging in near-freezing conditions, and 45 °C in hot weather (Seeley et al., 1985). Two species of *Melipona* in the Amazon exhibited ectothermy, with thoracic temperatures ranging from 32 to 38 degrees Celsius while the ambient temperature was close to 30 degrees Celsius (Roubik and Michener, 1984).

From these investigations, we may conclude that bees can adapt physiologically to withstand climatic change. Cooper et al. (1985) observed that under higher ambient temperatures, honeybee foragers in the desert return to their nests more often with a drop of nectar or water dripping from their proboscis. In addition, the chest temperatures of employees returning with fluids were at least 2 degrees Celsius lower than those returning with just pollen.

Honey bees were more sensitive to changes in relative humidity than were solitary bees. Some data from India indicate that light intensity is highly connected with Megachile's foraging activity, although humidity and temperature are rarely associated with *Megachile*'s or *Xylocopa*'s foraging (Heinrich and Buchmann, 1986). Similar to other bees, they were discovered to be very ectothermic (body temperature was higher than the

ambient temperature). However, hairy euglossine species lost heat mostly from the thorax by pumping the heated hemolymph towards the head, which was much greater than comparable bumblebees in temperate areas. In addition, chest feathering did not significantly slow heat loss from the torso at low wind speeds. In order to avoid overheating, certain tropical bees may rely more on slow flying, calm weather, relatively poor insulation, or periodic rest (Heinrich and Buchmann, 1986). Despite the larger heat generation of the flight muscles, somewhat hairless bees may raise their flight speed to maximize heat loss through forced convection (Heinrich and Buchmann, 1986). If we apply Berkmann's rule, it may explain why racial variants of a particular species are bigger and darker at higher altitudes and in colder regions. Larger animals have a lower surface area-to-volume ratio than smaller animals, so they radiate less body heat and stay warmer in cold weather (Ruff, 1994).

In other words, bee body temperature is influenced not only by the surrounding temperature, but also by elements like as metabolic heat generation, insulation, and heat loss by convection, radiation, and evaporation (Heinrich, 1993). All of these variables are influenced by body size and color. In a number of insect families, temperature excess in direct sunshine is mostly based on body size, although it becomes increasingly reliant on body color and size (Willmer and Corbet, 1981). These studies demonstrate that bigger insects absorb and release heat more slowly than smaller insects, yet reach greater temperature extremes. Small bees will warm up and cool down faster because convective heat loss is proportional to surface area, but they will not reach excessively high body temperatures. Because small organisms have a larger ratio of surface area to volume, they are better able to keep their body temperature stable at relatively low ambient temperatures. In the neotropics, centriole bees that are active during the hot and dry season tend to be smaller, less developed, and lighter in color than those that are active during the rainy season (Roubik, 1989).

Therefore, if climate change continues, the body sizes and colors of extant cold zone bees may diminish and lighten over time. In response to these alterations, the foraging behavior of bees may alter. Larger bees may begin foraging earlier in the day and visit blooms in the shade.

3. Plant-Pollinator Interactions

In addition to the present dangers given by climate change, bees and pollination are threatened by human causes such as the use of pesticides, the spread of diseases, and habitat degradation leading to the reduction of wild and managed plants, according to Grünewald (2010). In addition, Grünewald advised him to guarantee that conservation measures be established with care to avoid the extinction of economically valuable pollina-

tors such as honey bees. As these tactics are practical, political, economic, and scientific collaboration is required.

There is a paucity of data on how climate change influences plant-pollinator interactions (Walther et al., 2002; Visser and Both, 2005).

Hegland et al. (2009) investigated how climatic warming affects plant and pollinator phenology and distribution, which are characterized by temporal (phenological) and geographical (distribution) mismatches in plant-pollinator interaction. Increasing the temperature influences the plant's blossoming period and the pollinator's departure date. The sooner the plants bloom and the pollinators appear in response to a temperature increase, the higher the temperature. As a result of the impact of these incompatibilities on plant-pollinator interactions, the frequency of pollinator visits, pollen buildup, reproductive success, and population dynamics of plants might be affected. In contrast, nectar and pollen quantities, food accessibility, reproductive success and survival, and population dynamics from the pollinator's perspective.

Explanations for flying behavior may include the physiology of bees, the physiology of plant nectar release, and the overall quality of blooming and non-flowering sources. In addition to controlling when flight activity might be commenced or decreased, meteorological conditions influence the feeding behavior of bees in flower fields.

Changes in the global climate alter the pollination reciprocity of plants. Climate change may have a significant impact on bee fauna (Kuchlein and Ellis,1997), and the local loss of pollinators is recognized as a hazard to uncommon plant pollination mutualism.Climate change may have a significant impact on bee fauna (Kuchlein and Ellis, 1997), and local pollinator loss is recognized as a risk to rare plant pollination mutualisms. (Wall et al., 2003) found that global temperature change impacts the pollination reciprocities of Alabama skin flower in a distinct and more nuanced manner (*Clematis socialis*). This species is native to North America. Temperature is often the most influential climate factor on spring plant phenology (Menzel et al., 2001). The highly different for the clematis, the temperature regimes of 1996 and 1997, as well as the diverse bee species that serve as pollinators each year, highlight how pollination reciprocity may shift as a region's climate warms or cools (Wall et al., 2003).

The lack of a taxonomic trend in the data permits estimations of the wider British flora. Based on 16% of species that flowered considerably earlier in the 1990s, 150 to 200 species may currently bloom in the UK on average 15 days sooner than in the very recent past (Fitter and Fitter, 2002). In the majority of instances, issues will develop apart from the intricacy of species interactions and the varying sensitivity of species to

changing environmental circumstances. Some species are able to rapidly adapt to new environments and compete with others. Numerous studies demonstrate that pollinators influence the reproductive success of target plant species because climate change may have an effect on pollinators, hence altering plant reproductive success.

The ozone layer filters the sun's most damaging UV energy. Ozone depletion refers to the observed reduction of ozone in the stratosphere during the last fifty years. Increasing UV radiation will have negative effects on the physiology and genetics of animals and plants. Additionally, some of the plant's blossoms reflect UV light. UV-A reflection serves as a nectar guide for bees (Dyer, 1996). As with humans, bees have trichromatic color vision, but the three major hues are skewed toward shorter light wavelengths (Frisch, 1967). Thus, bees sense UV as a distinct hue, but the dark orange-red band's longest wavelengths are not perceived. The European honey bee has the greatest sensitivity to the difference between ultraviolet and green. Therefore, flowers with UV nectar cues are most alluring against a green leaf backdrop. Nectar guides are regions of strong UV reflectivity on flower tops that attract and lead bees to nectar (Roubik, 1989).

Morandin et al. (2002) found that greenhouses with UV+ coating were less impacted by the loss of bumblebees due to ventilation than greenhouses with UV- coating. The application of UV– coatings has a good effect on bees since UV+ irradiation increases color contrast. A phototoxic response to UV+. Dye and Chittka (2004) demonstrated that bumblebees can use their visual systems to effectively look for food in conditions devoid of UV light. If differing UV radiation reaches the blooms as a result of ozone depletion and the light's wavelength changes, the bees' nectar guidance may get muddled. If this occurs, it will be more difficult for them to discover nectar and pollen sources.

Although we used honey bee literature to investigate the unique effects of climatic conditions on honey bees, similar effects may apply to other social pollinations. Only a few alternative pollinator species have been successfully cultivated. It is essential to develop protection methods for their native populations. If pollinator variety is vital to agriculture, we must understand how to manage plant species in the wild. Due to climate change, we should endeavor to establish alternate management strategies. We understand how to maintain honeybees and cultivated bees. If plants bloom early or late, we can regulate the honey bee colony population. However, we cannot accomplish this for populations of wild pollinating bees.

According to Le Conte and Navajas (2008), the biodiversity and adaptability of honey bees are crucial to the climate change process. Since

a result, honey bees will have to adjust to several dangerous infections, parasites, and new pressures in the event of a change in temperature and environmental circumstances, as honey bees have a high genetic variety that is distributed globally. For instance, Cornelissen et al. (2019) reported in their study that global warming encourages the biological invasion of the tiny hive beetle species, which has the potential to severely harm globally significant pollinator species. Consequently, this genetic variety is the driving factor enabling adaptation to potential new biotopes. On the other hand, they noted that climate-related pressures would pose certain threats to the species and that genetic variety in honey bees can be conserved if humans design their habitats accordingly.

CONCLUSION:

In summary, it is clear that social bees will have a greater survival rate than solitary bees as a result of the continuing climate change. It is possible that the bees' bodies may get smaller and their colors will become lighter. As plant variety declines, the success of specialist bee species on common plant species will increase. Reduced plant variety may lead to an expansion in the ranges of insect pests and diseases over time. Consequently, growers may use more and more potent pesticides. Pesticides will adversely impact the dynamics and survival of pollinator populations. This high level of genetic diversity of bees and the species, subspecies, and ecotypes of bees that are essential to the global pollination and breeding program will need conservation strategies. To conserve honey bees, it is necessary to continue breeding, management, and monitoring programs to reduce migratory beekeeping, improve the protection zones of local native honeybee populations, avoid chemical exposure, and develop bees resistant to diseases and pests. It is essential, for the conservation of solitary bees, to integrate wild blooming plant regions in monoculture agricultural areas and to preserve these areas from chemical exposure. In urban parks and gardens, care should be made to establish bee-friendly plants with nectar and pollen and to avoid using pesticides.

Compliance with Ethical Standard

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CHAPTER 4

RESTORATION, CONSERVATION AND GOVERNANCE OF NATIVE CRAYFISH POPULATIONS

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1. INTRODUCTION

Freshwater crayfish are important for many reasons. Ecologically, freshwater crayfish can have significant impacts on the ecosystem because of their importance in the food webs in their environment, as they can be the most intensive aggressive invertebrate group in the aquatic environment. Economically; crayfish are one of the most valuable species harvested from freshwaters and are consumed as "luxury food". Socially; they are traditionally consumed in some countries (i.e., in Scandinavian countries) and provide economic incomes to fisheries sectors (Hogger, 1988; Momot, 1995; Danilović et al., 2022). However, many European native crayfish species populations suffered considerable damage due to reasons such as overfishing, environmental pollution, alteration of their habitat and especially crayfish plague disease caused by oomycet (*Aphanomyces astaci*) (Martín-Torrijos et al., 2018; Jussila et al., 2021; Martínez-Ríos et al., 2022¸ Mirimin et al., 2022).

According to a report published in 2015, one-third of the world's freshwater crayfish populations are at risk of extinction. A considerably group of researchers from Austria, USA, Ireland, Mexico, England and Australia led by the London Zoological Society, assessed the risk of extinction of 590 freshwater crayfish species in the world based on the IUCN Red List categories. 32% of the global crayfish species were listed as "danger of extinction" by the team; this figure was a much higher figure than most animals living in the sea or on land. It is unlikely that this high risk of extinction will help in the fact that only a small proportion of the global crayfish populations are covered by conservation sites (Richman, 2015; Bland, 2017; Danilović et al., 2022; Hudina et al., 2022). For example, the populations of *Austropotamobius pallipes* in Europe declined by 25-49% by the 2000s.

In Eastern England, approximately 77% of the native crayfish populations declined over the last decades. primarily to be because of non-indigenous crayfish species and crayfish plague (*Aphanomyces astaci*). In France, the situation for three native crayfish species of concerned: *Austropotamobius torrentium* and *Astacus astacus* were very near to endangered, and *A. pallipes*, with mortalities observed in 47 habitats, coukd now only be observed in the upper sides of the basins. In Belgium, *A. astacus* disappeard from Flanders. On the other hand, *Pontastacus leptodactylus* and *Pacifatacus leniusculus* have slowly expanding their distributions and eredicating *A. astacus* in Wallonia (Holdich et al., 2009). Moreover, populations of non- indigenous crayfish species continue to increase in Europe (Kaldre et al., 2020). Therefore, it can be stated that non-indigenous crayfish species can eliminate entirely native crayfish populations in the next few decades unless governance actions are done to conserve them. For these reasons, it is

necessary to restore and manage the existing populations of crayfish and to work to recover their numbers, as well as the populations that are destroyed or damaged by any cause. Therefore, the restoration, conservation and governance of native crayfish populations is very crucial.

Invasive non-indigenous crayfish can have reinforced significantly negative effects on the richness of indigenous (native) species and can alter aquatic habitats in some ways. For example, invasive non-indigenous crayfish outcompete native crayfish for shelter and food (Harlıoğlu and Mişe-Yonar, 2007; Harlıoğlu and Farhadi, 2017). Moreover, in freshwater habitats, the diversity and abundance of macroinvertebrates are decreased by non-indigenous crayfish. Some non-indigenous crayfish species (i.e., *Procambarus clarkii*) significantly change structure of waterbody bottom/side through burrowing activities (Ruokonen et al., 2018). However, there are two main reasons for the restoration of crayfish populations. The first reason; it is the restoration of the disappearing populations due to the crayfish plague (causative agent *Aphanomyces astaci)*. For example, approximately 90% of native European crayfish populations disappeared or are at risk of extinction due to crayfish plague outbreaks (Freemana et al., 2010; Jussila et al., 2015). Similarly, *Pontastacus leptodactylus* was one of the water products of importance for Turkey's economy before the crayfish plague reached the waterbodies of the country. In Turkey, the production of *P. leptodactylus* increased rapidly in the early 1960s, reaching an average of 4500 tons per year between 1978 and 1984. However, the native crayfish production of Turkey decreased dramatically due to the disease first seen in 1984 in Işıklı (Çivril) Lake and then spread to other aquatic environments (Erençin and Köksal, 1977; Köksal, 1988; Aydın et al., 2012; Güner and Harlıoğlu, 2009; Harlıoğlu and Harlıoğlu, 2009).

The second important reason of their disappearance is the environment changes: freshwater habitats are directly or indirectly under strong negative anthropogenic pressure for various purposes (Gydemo, 1994; Holdich, 1999; Gutierrez-Yurrita et al., 1999; Ackefors, 2000; Ruokonen et al., 2018). Revitalization of native crayfish production could be achieved through the restoration and conservation of damaged populations. Therefore, this review discusses the importance of restoration of crayfish populations and the work to be done for the conservation and governance of native populations.

2. STRATEGIES FOR RESTORATION OF CRAYFISH POPULATIONS

One of the easiest methods of restoration is to replace native crayfish species with a new "equivalent" crayfish species. However, it is not known exactly what consequences this kind of crayfish stocking will cause in the

future. Scientists, however, do not support replacing a native species with a non-indigenous species because of the inability to demonstrate a fully biologically and ecologically compatible environment after such replacements (Gydemo, 1994). On the other hand, there are many examples showing that such stocks are profitable economically. As a result, in the crayfish restoration, when it comes to replacing the native species with a non-indigenous crayfish species, large-scale economic problems are becoming more and more serious, leading to the idea that "if a species does not have economic benefit, this species can be ignored" (Ackefors, 1999).

In European countries, there were two important crayfish species in the restoration of crayfish populations until the early 1990's. These species were *Pacifastacus leniusculus* and *Astacus astacus* (Avault, 1993; Rogers and Holdich, 1995). When called for restoration with *P. leniusculus*, crayfish stocking for economical purposes, and restoration with *A. astacus*, it was firstly understood that these populations were protected, supported and indirectly stocked with crayfish for economic purposes (Gydemo, 1994). Apart from these two species, *P. leptodactylus*, a native crayfish species of Eastern Europe was also stocked for commercial purposes for natural water resources in Europe (Köksal, 1988; Holdich, 1999; Taylor et al., 2019). However, today, it is forbidden to populate waterbodies with non-native crayfish species and *P. leniusculus*. Because of the fact that *P. leniusculus* is on the EU list of invasive species of EU concern (URL, 1; Hudina et al., 2022).

Before a crayfish population is restored, it is necessary to decide whether to replace the native species in the population with another crayfish species or to repopulate the population with the same species. If the purpose of the restoration is to generate economic income, then the best choice to do; is the stocking of *P. leniusculus* or another North American crayfish species, taking into account the infinite oomycet disease setting for that water environment, complete disappearance of the native crayfish species from the environment, and other risks of economic deterioration. However, it should not be forgotten that prices have fallen, especially in the years when the number of crayfish growers is increasing and crayfish production is high.

The decision to stock a crayfish population that has been damaged by the fungal disease with the same species (native species) is very difficult because of the high possibility that the fungal disease is still present in the waterbody (Söderhall et al., 1977; Oidman, 2000). Possible factors that cause the decrease of crayfish stocks other than fungal diseases are:

1. Habitat degradation: the use of lake or pond water as irrigation water, destruction of forested areas, changes in water levels (i.e., due to cli-

mate changes, changes in water level of underground and waterbodies), excavation and filling works on the ground of water environment to establish land areas, sedimentation, destruction of vegetation structures (harvesting), harbour and dam constructions (Harlıoğlu, 1996; Holdich, 1988; Taylor et al., 2019).

2. Water quality deterioration: pollution, for example; the reduction of the pH of the water with acidic rains, the discharge of heavy metal wastes into the water source, the plant fertilizers coming from the agricultural areas, the eutrophication enhancing and oxygenous substances in the water caused by the forest areas and settlements (Gydemo, 1994; Gutierrez-Yuritta, 1999; Danilović et al., 2022).

3. Competition, predation and disease transmissions among species can increase with an economically important crayfish species stocking. In addition to the crayfish plague, diseases caused by the genus *Saprolegnia* can cause an increase in crayfish mortality. In addition, these diseases and parasites are more visible and have sinergistic effect during periods when environmental conditions and water quality are poor (Westman, 1995; Oidman, 2000; Souty-Grosset et al., 2016).

4. Over-fishing: changes can occur in the ecosystem depending on changes within the species and between the species as a result of over-fishing (Westman, 1995; Harlıoğlu, 1996; Holdich, 1988; Krieg and Zenker, 2020).

The populations of native crayfish species in Europe are now threatened by one or more of these factors. Besides these factors, the European native crayfish species, which are less aggressive than the introduced non-native invasive crayfish species (especially *P. leniusculus* and *Procambarus clarkii*) stocked in Europe are less fecund and are growing slower, and are more difficult to adapt to environmental conditions, makes the selection of non-indigenous species more attractive in the selection of species to be made in the restoration of their populations (Avault et al., 1986, Hogger, 1986, Holdich et al., 1995, Holdich and Domaniewski, 1995, Huner and Avault, 1979; Theissinger et al., 2021).

3. RESTORATION METHODS

Today, there is no restoration method applicable for every crayfish population. A restoration method suitable for one population may not be suitable for another population. For this reason, ecological, economical and genetics of populations, and even social factors need to be investigated in detail before any restoration. When a restoration method is determined, a general scheme that should be applied is as follows (Lodge and Hill, 1994: Holdich, 1999; Burba, 1993; Henttonen, 1996; Skurdal, 1994; Hefti

and Stucki, 2006; Závorka et al., 2020).

1. Determination of the population characteristics (i.e., growth rate, sex ratio, length-weight relations, condition factor, meat yield, breeding season, egg production, feeding habits and shelter usage) and environmental factors that influence crayfish population (i.e., water quality, climate conditions, pollution). In addition, when this determination is carried out, it is necessary to consider high numbers of crayfish obtained from the population should be tested (Lodge and Hill, 1994: Holdich, 1999; Souty-Grosset et al., 2016; Hudina et al., 2022).

2. After determining the characteristics of crayfish species population and the environment, determine which of these properties (i.e., economic importance, fecundity, growth rate, aggressiveness behavior) is important and admirable in the case of restoration (determination of the species to be stocked) and ultimately making the appropriate decision (Skurdal, 1994; Henttonen, 1996; Adams and Marks, 2016). In addition, the correct determination of which population can be obtained from the most suitable crayfish for stocking and whether juvenile or adult crayfish will be used in stocking will positively affect the success of the restoration (Kozák et al., 2011).

3. Will a previously crayfish-containing water source be stocked with the same species or with a non-indigenous species not previously found in that water source, so that it will be able to reach the productive status of previous years? What changes (i.e., physical, biological and ecological) will occur to the water resource when a non-indigenous invasive crayfish species is stocked? How will the fish fauna in the water resource be affected by the new crayfish stocking? The answers to the questions should be taken into account (Holdich, 1999; Hefti and Stucki, 2006; Adams and Marks, 2016; Silknetter et al., 2020).

4. Selection of the species and method of crayfish stocking: the above questions should be answered and the species selection and crayfish stocking method should be determined taking into account the entire ecosystem, not just the area where only one water environment is to be stored. The actions should be in accordance with the legislation (law regulations). For example, in majority of European countries *P. leniusculus* has been prohibited from stocking into any water source to prevent it from carrying crayfish. Similarly, in Europe, most European countries, *P. clarkii* has also been prohibited from stocking new environments such as *P. leniusculus* because it damages the set and water structures, especially in dam lakes, as a hideout (Holdich, 1988; Souty-Grosset et al., 2016; Silknetter et al., 2020).

On the other hand, before determining the type of crayfish to be used in the restoration, consultation with fish cooperatives, fishermen, farmers using agricultural fertilizers, institutions that legally protect the environ-

ment, industrial enterprises that process crayfish and people who consume crayfish, and deciding on the selection of the most suitable crayfish will ensure the best results.

4. CONSERVATION AND GOVERNANCE OF NATIVE CRAYFISH POPULATIONS

An essential part of any conservation and governance strategy is knowledge of the crayfish's range of distribution in the aquatic environment; populations size; threats to crayfish survival; presence and/or absence of non-indigenous crayfish species in the same habitat and crayfish plague, what changes are expected to affect the environment over time (Holdich et al., 2002; Theissinger et al., 2021; Sutherland et al., 2022)?

The following actions should be taken to protect and governance native crayfish populations:

1. Crayfish catching should be prohibited except certain periods of the year. In addition, mated females, egg bearing females, and females carrying first and second stage juveniles should never be caught.

2. Crayfish catch methods and tools should be restricted, controlled and allowed only to catch legal size of crayfish. In addition, in order to eliminate the possible contamination of pathogens (such as *A. astaci*), catch tools should not be used for hunting in different habitats, if possible, and disinfected if not possible.

3. The situation of crayfish populations against crayfish plague should be identified and declared by official institutions that are "in danger", "endangered populations" or "healthy populations", and action plans should be prepared to increase their production. In addition, **populations** with crayfish plague outbreak records should be labeled on the map and the sale of crayfish caught from these populations should be controlled. All of the fishing equipment used in these populations where crayfish plague was recorded should be disinfected.

4. Ecological, immunological, genetic and aquaculture investigations of species in populations should be carried out to increase the production of crayfish species.

5. Breeding and rearing of economically important crayfish species should be supported and increased in captivity. Produced juveniles should be stocked into freshwater resources where crayfish were previously found.

6. New crayfish stockings should be made from healthy crayfish populations to damaged populations and the results should be checked.

7. The number of shelters, which is of great importance in hiding the crayfish on the ground, should be increased to reduce the rate of cannibalism. In addition, crayfish have many predators, including fish, amphibians, reptiles, birds, and mammals. Some of the most common land predators of crayfish are mergansers, turtles, herons, kingfishers, muskrats, snakes. Taking precautions against crayfish predators, especially in populations of economic importance, will increase production.

8. Zooplanktonic organisms, which are particularly important in the feeding of juvenile crayfish, should be enriched in the aquatic environment. Supplementary food should also be supplied to increase reproduction.

9. While it is not possible to completely eradicate crayfish plague in large lakes and rivers, status of crayfish population can be improved by re-stocking disease-free crayfish and disinfection catching tools and other equipment.

10. Prevention of stocking of non-indigenous crayfish species in natural water sources, prohibition of non-indigenous crayfish species moving from one habitat to another, if non-indigenous crayfish species are in any environment, preventing them from escaping to other habitats.

11. The introduction of non-native crayfish species into natural water sources, their movement from one freshwater resource to another, and their escape to other freshwater resources have to be prevented. In addition, informing public, administrations and fishermen about the problems that may arise from the stocking of non-indigenous crayfish species.

12. Control and management of non-indigenous crayfish species should be carried out scientifically. Although there have been few studies done for this purpose, few practitioners have provided relatively little concrete data on what decisions can be made. However, it is considered possible to successfully implement threat reduction attempts where sensitive native crayfish populations are clearly at risk. The following techniques can be used as part of attempts to control non-indigenous crayfish populations (Souty-Grosset et al., 2004; Stebbing et al., 2014; Krieg and Zenker, 2020):

Biological control (predator-prey interactions), use of pesticides to non-indigenous crayfish species, removal of crayfish from population by catching, establishing physical or electrical barriers, use of pheromonal attractants (such as sex pheromones and feeding cues) in crayfish trapping, sex ratio manipulation (Removal of egg-laying female crayfish and relatively small male crayfish by selective hunting, and leaving relatively big males in population. Reducing egg production will result in a decrease in population growth. It is also known that large male crayfish suppress

the daily activities of juveniles and small crayfish and are cannibalistic on them), releasing enormous numbers of sterilized male crayfish into the population.

5. CONCLUSION

Crayfish populations in Europe have been devastated by the crayfish plague for about 150 years, and crayfish production values have therefore decreased considerably. Some invasive North American crayfish were deliberately stocked in European water supplies to compensate for production declines and economic losses. However, these stockings caused mass mortalities of native crayfish, and as a result, the amount of crayfish production decreased further rather than increased. Therefore, there has a need to restore and sustainably govern many natural crayfish populations in Europe.

Invasive crayfish species *(i.e., Pacifastacus leniusculus*, *Orconectes limosus*, *Orconectes virilis*, *Orconectes rusticus*, *Procambarus clarkii*, *Cherax quadricarinatus*, *Cherax destructor*, *Faxonius rusticus*) are aquatic creatures that cause the most damage to freshwater resources globally when they are transferred from their environment to other environments. Therefore, their introduction from their natural range to new habitats has to be banned for any reasons. In addition, due to their colors some invasive crayfish species are imported for aquarium keepers. On the other hand, the importation of invasive species for use in the aquarium sector causes these species to reach the natural water resources of the countries they are imported from. Such imports should also be strictly prohibited in order to protect and manage native crayfish populations and ensure their sustainable use.

In order to obtain good results from the restoration and governance of crayfish populations, it is thought that a national policy should be determined that includes authorities, managers, fishermen, social media and the public. In addition, science-based advisories that non-indigenous crayfish species harm native ecosystems and native crayfish populations should be taken with the most serious caution, regardless of commercial concern. In parallel with the restoration of crayfish populations, sustainable use of water resources will be ensured. Moreover, effective management of populations of both endangered native and invasive non-indigenous crayfish requires knowledge of their distribution, ecology and population biology within the country, monitoring of existing populations, and identification of newly established populations.

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CHAPTER 5

STRATEGIC ANALYSIS OF BEEKEEPING ENTERPRISES; A CASE STUDY OF VAN PROVINCE

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1. INTRODUCTION

Agricultural sector has a critical importance compared to the other sectors in terms of its social, political, technical and economical characteristics. The rapidly increasing population in the world and also in our country comes up with many problems but adds great value to agricultural production. In addition to the function of agriculture in meeting the basic needs of people, it emerges as a sector of great importance in the economy thanks to its contribution to employment, agriculture-based industry, foreign trade and national income.(Arısoy and Oğuz, 2005). Van province has a very rich natural flora in terms of feasibility of beekeeping activities. This richness has a great importance when it comes to add varieties in alternative production activities. In the rich vegetation of Van province with dense milk vetch and thyme species, which is preferred by hundreds of mobile beekeepers in each production season, there are a total of 283 plants, 253 natural and 30 cultivated plants, which are important for beekeeping, and 25 of these plants are endemic. In the light of the information above, the province plays an important role in beekeeping activities. (Öztürk et al., 2017) Considering the potential of Van province, the current situation of the beekeeping sector is seen as an area open for development and improvement. In particular, the diversification of beekeeping products and their transformation into byproducts will be very important in terms of its contribution to the sector. (Çakal, 2013).

In this study, we have examined the businesses that benefited from the support under 302-2 Beekeeping and Production, Processing and Packaging of Bee Products sub-sector within the scope of the IPARD program established in order to create capacity for ensuring sustainable development by taking into account of the priorities of Turkish policies before the enrollment into EU and to ensure harmonization of the businesses with EU Standards. Beekeeping enterprises in the region cannot benefit from environmental conditions and resources sufficiently. The biggest inadequacy here is the inability to determine the right policies and strategies, and this has been stated in many literature (Narayan, 2000; Dyson, 2004; Önder and Polat, 2004; Akça et al., 2006; Durgun, 2007; Yüksel and Dağdeviren, 2007; Kansız et al., 2008; Hussain et al., 2009; Büyükalaca et al., 2009; Markovska et al., 2009; Tutar et al., 2009; Toksoy et al., 2009; Vermeire and Gellynck, 2009; Karamürsel, 2010; Subaşı et al., 2010; Büyükalaca et al., 2011; Hussain et al., 2011, 2012; Demirtaş, 2013; Haryadi, 2014; Jaleel et al., 2014; Uysal and Subaşı, 2014; Subaşı et al., 2015). In this study, it is aimed to evaluate the existing structure and create strategies for the process of making the IPARD program and the role of beekeeping enterprises in the country's growth and development more prominent in the province of Van, which has a very important place in honey production in Turkey.

2. MATERIAL AND METHODOLOGY

The main material of the research consists of the data collected from beekeeping enterprises receiving the IPARD I program support in Van province through questionnaires. Also: In addition to this data, publications and web sites of relative public institutions in the research area and Ministries of Agriculture of Turkey and EU countries, Turkish Statistical Institute (TSI), the Food and Agriculture Organization (FAO), the World Bank (WB), European Union Statistical Institute (EuroStat) and previous research findings and published secondary data were used.. Van province having nearly 3% of honey production in Turkey (TSI, 2018) and 4% of total hives has been selected as research area. In Van province, 94 beekeeping enterprises that received IPARD support were taken as samples according to the full inventory method. The research data were compiled by the researcher according to the survey technique from the sample enterprises, and the data belong to the production period of 2016.

2.1. The Methods Used in Developing Enterprise Strategies

2.1.1. SWOT and SOR Analysis

SWOT Analysis is a method to systematically identify various factors to formulate corporate strategies. This analysis is based on logic that maximizes strengths and opportunities, while revealing results that can minimize weaknesses and threats. The strategic decision-making process is always associated with the development of the company's vision and mission, objectives, strategies, and thus policies. Therefore, organizations that want to make strategic planning should analyze their current strategic factors (strengths, weaknesses, opportunities and threats) efficiently. This is called as situation analysis and the most popular model for situation analysis is SWOT Analysis.(Rangkuti, 2005) In the literature, SWOT is an instrument of superiority. The SWOT contains an outline for strategies based on found strengths and weaknesses, and promising combinations of opportunities and threats.(Rauch, 2007)

Table 2.1 *SWOT matrix*

	Strengths (S)	Weaknesses (W)
Opportunities (O)	Strategy (SO) Developing strategies using strengths in order to benefit from opportunities	Strategy (WO) Developing strategies minimizing weaknesses by benefiting from opportunities
Threats (T)	Strategy (ST) Developing strategies using strengths in order to cope with threats	Strategy (WT) Developing strategies minimizing weaknesses in order to avoid threats

The results of the SWOT analysis consist of four alternative strategies that can be used as a substantive assessment by standard enterprise management. (Rangkuti, 2005):

1- SO Strategy (Strengths - Opportunities). In this strategy, you use internal potential of the enterprise to acquire external opportunities. Therefore, if enterprises has a weakness it should deal with the weakness. In case of that the enterprise faces a threat, then the enterprise should try to avoid it and to focus on the opportunities.

2- WO Strategy (Weaknesses - Opportunities). This strategy aims to minimize internal weaknesses of the enterprise in order to benefit from external opportunities. In some cases, enterprises face with challenges in terms of benefiting from opportunities due to internal weaknesses. For this reason, efficiency of the management depends on the how the enterprise use the strategy and overcome the internal weaknesses.

3- ST Strategy (Strengths - Threats). Thanks to this strategy, the enterprise aims to avoid and reduce the effects of external threats.

4- WT Strategy (Weaknesses - Threats). This strategy is a tactic for survival by reducing internal weaknesses and avoiding threats. Faced with several internal weaknesses and external threats, a company must struggle to survive, recognizing the danger.

2.1.2. SOR Analysis

Also called Strategic Orientation Round (SOR) or formulation strategy, it is generally considered as a set of strategic methods or long-term planning. Before the Strategic Guidance starts, the SWOT tables and SOR matrixes are given to each of the participants in accordance with the figure

3 above, and the SOR method consisting of the following steps is applied. (Gellynck and Vermeire, 2009; Haryadi et al., 2019; Karamürsel et al., 2020)

Participants are asked for rating each cell considering the priorities of the beekeeping sector ("3-the most important" to "0" the least important") and final versions of SOR matrixes are created. Rating system is associated with O's and T's and evaluated in terms of for which S's and W's it can be useful. Here:

3 = very important

2 = important

1 = slightly important

0 = important/not relevant

In addition to this, maximum of 12 votes can be distributed for each O and T. It should be noted that this may only be the maximum, not the minimum amount allowed. Strategy, which is the set of goals, policies and plans to achieve the determined aims, is about the future and is the series of decisions that the enterprise has made to reach the optimum. While determining their strategies, enterprises should develop a flexible organizational structure that can adapt to the changes in these dynamics by observing the internal and external environment dynamics, as well as analyzing their current situations. Therefore, enterprises should determine the most proper strategies for their organizational structures. In today's intense competition and rapidly changing environment, enterprises should analyze their basic advantages well and develop these advantages in order to maintain their existence in the market and to provide competitive advantage. The products, service level and activities delivered by the enterprise should be able to meet the expectations to a significant extent.

Table 2.2 *SOR interpretation stages*

SOR matrix consists of high-volume information. After the process has been completed, region representatives are responsible from explaining it and derive strategic goals from it. The partner may interpret the schemas in different ways. However, it is critical to monitor the scores.	What does it mean?	What will we do with it?
Total score per each S, W, O, T.	How important is it that the S's, W's, O's, T's are different?	The strategy aims to make the maximum benefit from the external factors. Therefore, please create the strategy around the 2 or 3 most important O's & T's.
Total score per each combination.	How important is it that O or T is associated with W?	Improve the strategic goals related to combination with the highest score.
Total score per each quarter.	What are the general expectations about the future?	High S-O: Go for it, you have a high change High S-T: strengthen your guard, we have the power to overcome the threats. High W-O: Clear the decks or redirect, work on your weaknesses to take advantage of the opportunities available. High W-T: Crisis situation, threats are serious and we don't have the required tools to deal with these.

Reference:(Gellynck and Vermeire, 2009)

3. 3.1. 1. CONCLUSION OF THE STUDY AND DISCUSSION

Developing Strategies in Examined Enterprises, SWOT and SOR Analysis

SWOT Analysis

The results of the SWOT analysis consist of four alternative strategies that can be used as a substantive assessment by standard enterprise management (Rangkuti, 2005).

Table 3.2 *Internal factor analysis of beekeeping sector in Van province*

Internal Strategic Factors
1. Strengths
Having lands in the status of meadows and pastures suitable for honey yield and having large flora areas
The accumulation of experience brought by the old culture of beekeeping
The presence of associations related to beekeeping and high resident population who can do beekeeping in the region
To be able to start beekeeping activities with a low budget
Creating of database by giving enterprise and producer numbers to beekeepers by existing unions
Increasing efforts of Beekeeper Unions and Cooperatives for honey marketing
Thanks to the easily accessible experience of East Anatolia Agriculture, Livestock and Food Fair held in Van ,creating an opportunity for promoting Van honey
High number of hives
Demand for regional honey
Start of the development of professional beekeeping
Uncontaminated soil, water and environment
2. Weaknesses
Insufficient queen production and breeding problems
The low level of education of beekeepers, the inadequacy of training activities in this context and their lack of eagerness for innovations.
Lack of diversity in the production of bee products and not knowing the importance of byproducts other than honey
The possibility of rapid spread of epidemic diseases due to the colonized life of bees

Not preferring the laboratory analysis process and high analysis fees
Differences in the honey quality
Mixed honey harvesting
Failure to serve the plateaus to production due to terrorism
Instability in the market and insufficiency in marketing
Beekeeping is not seen as a profession by the local people

Table 3.3 *External factor analysis of beekeeping sector in Van province*

External Strategic Factors
1. Opportunities
The increase in honey demand due to the rise in consumer awareness about quality of bee products and the breakfast culture in Van province
Ability to produce honey consciously as a result of effective training for producers and popularizing activities
To increase organic honey production and in accordance with this creating new production sites
Increasing efforts regarding brand development
Encouragement of beekeeping by the state and availability of state supports
Presence of a beekeeping research and application center at Van Yüzüncü Yıl University and giving practical beekeeping education
Accelerating competition due to increase in the number of enterprises processing honey
Value of the bee products
To increase the added value of bee products converting them to byproducts.
The province of Van is on the international trade route.
2. Threats
The emergence of unfair competition and the victimization of the Turkish beekeeper due to border trade and illegal export of poor-quality honey to the research area
Low competitiveness of producers
Ongoing fake honey and synthetic honey problems

Insufficient R&D studies for bee products

Not to be able identify the origin of honey in case of mixed harvesting

Insufficient statistics of honey (extracted and comb honey) and other bee products

Loss of quality of honey in case of mixed harvesting

Uninterest towards science, technology and research

The monopoly of beekeepers by powerful companies

Unavailability of mobile beekeeping within the province due to terrorism

In the first stage, the factors affecting the characteristics of the sector and its environment, respectively, were determined. Then, the officials of government institutions, non-governmental organizations and educational institutions, who have a leading and controlling position in the sector, gave scores between 1 and 10 points for each feature. The severity was determined by the participants scoring the specified number of elements in the matrix from the most important to the least important, taking the arithmetic average of each weakness and strength, threats and opportunities. In rating, the most important element is 10 and the least important element is 1. Internal and external factor analysis of the beekeeping sector in Van is shown in Table 3.4.

Table 3.4 *Interpretation of the main strengths, weaknesses, opportunities and threats of the beekeeping sector in Van province and rating before SOR analysis*

No	Internal Strategic Factors	Score
1.	**Strengths**	
	• Having lands in the status of meadows and pastures suitable for honey yield and has large flora areas	**9,05**
	• Uncontaminated soil, water and environment	**8,41**
	• Demand for regional honey	**8,32**
	• The accumulation of experience brought by the old culture of beekeeping	**7,95**
	• Creating of database by giving enterprise and producer numbers to beekeepers by existing unions	**7,73**
2.	**Weaknesses**	
	• Failure to serve the plateaus to production due to terrorism	**9,36**
	• Not preferring the laboratory analysis process and high analysis fees	**8,50**
	• Instability in the market and insufficiency in marketing	**8,45**
	• The possibility of rapid spread of epidemic diseases due to the colonized life of bees	**8,41**
	• Insufficient queen production and breeding problems	**8,36**
3	**External Strategic Factors**	
	Opportunities	
	• Value of the bee products	**8,27**
	• Presence of a beekeeping research and application center at Van Yüzüncü Yıl University and giving practical beekeeping education	**8,00**
	• Encouragement of beekeeping by the state and availability of state supports	**7,95**
	• Increasing efforts regarding brand development	**7,50**
	• To increase organic honey production and in accordance with this creating new production sites	**7,41**

	Threats	
4	• Ongoing fake honey and synthetic honey problems	**9,41**
	• The emergence of unfair competition and the victimization of the Turkish beekeeper due to border trade and illegal export of poor-quality honey to the research area	**9,00**
	• Insufficient R&D studies for bee products	**8,86**
	• Unavailability of mobile beekeeping within the province due to terrorism	**8,82**
	• Low competitiveness of producers	**8,50**

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3.1.2. SOR Analysis

Participants are asked for rating each cell considering the priorities of the beekeeping sector ("3-the most important" to "0" the least important"). The final version of SOR matrix is shown in Figure 3.1.

RATING			OPPORTUNITIES					THREATS					TOTAL
			O1	O2	O3	O4	O5	T1	T2	T3	T4	T5	
			Value of the bee products	Presence of a beekeeping research and application center at Van Yüzüncü Yıl University and giving practical beekeeping education	Encouragement of beekeeping by the state and availability of state supports	Increasing efforts regarding brand development	To increase organic honey production and in accordance with this creating new production sites	Ongoing fake honey and synthetic honey problems	The emergence of unfair competition and the victimization of the Turkish beekeeper due to border trade and illegal export of poor-quality honey to the research area	Insufficient R&D studies for bee products	Unavailability of mobile beekeeping within the province due to terrorism	Low competitiveness of producers	
STRENGTHS	S1	Having lands in the status of meadows and pastures suitable for honey yield and has large flora areas	50	44	45	20	14	42	47	22	54	36	374
	S2	Uncontaminated soil, water, and environment	26	39	30	26	19	24	28	20	40	18	270
	S3	Demand for regional honey	45	29	695	35	25	32	47	733	26	52	43	369
	S4	The accumulation of experience brought by the old culture of beekeeping	13	23	19	33	21	22	18	31	26	16	222
	S5	Creating of database by giving enterprise and producer numbers to beekeepers by existing unions	14	14	26	35	15	19	16	13	23	18	193
WEAKNESSES	W1	Failure to serve the plateaus to production due	48	24	43	12	33	49	51	27	52	37	376
	W2	Not preferring the laboratory analysis process and high analysis fees	39	44	26	40	14	51	46	35	48	34	377
	W3	Instability in the market and inadequacy in marketing	16	8	652	21	18	17	53	35	915	18	47	22	255
	W4	The rapid spread of epidemic diseases due to the colonized life of bees	15	43	28	14	16	14	19	26	13	23	211
	W5	Insufficient queen production and breeding problems	13	44	41	16	19	53	45	46	29	42	348
TOTAL			279	312	314	249	193	359	352	264	384	289	2995

Figure 3.1. *Strategic orientation matrix of beekeeping sector in Van province*

It is given importance to include participants from different sectors. Each participant has been requested to examine and discuss the strategies given to them, to determine the actions to be taken within the scope of these strategies and the set of the activities to be carried out in order to carry out these actions and specify by whom, in cooperation with whom and when these activities will be carried out. Afterwards, the strategies for the development of the beekeeping sector in Van Province were determined as follows by bringing together the studies of all groups within an objective framework and adhering to the suggestions from the groups.

1. Taking advantage of that the Van province has eligible lands in the status of meadows and pastures that contain wide flora possibilities in terms of honey yield and that the bee products produced in these areas are valuable.

2. Since the honey of the region has a demand, to ensure that beekeeping is encouraged by the state, there are state supports and these supports are directed to eligible meadow and pasture lands that contain wide flora characteristics in terms of honey yield of Van province.

3. Providing practical beekeeping trainings by the beekeeping research and application center at Van Yüzüncü Yıl University for aiming the weakness of not preferring the laboratory analysis process and high analysis fees.

4. To resolve insufficient queen bee production and breeding problems, increasing the number of enterprises producing queen bees with the state supports and the studies of the beekeeping research and application center at Van Yüzüncü Yıl University.

5. Beekeepers are suffered from terrorism and face with security problems. Illegal border trade poses a threat for the future.

CONCLUSION AND RECOMMENDATIONS

In the study, after determining the general structures of the enterprises, it is aimed to evaluate the existing structure with SWOT and SOR analysis and to offer persistent solutions to the problems encountered in the examined enterprises. Research results in the region show that the beekeeping sector has a need for significant structural change. Beekeping activities has become a profession as a main source of income. It must be taken into account when developing policies regarding the sector, and beekeeping should now be seen as a profession, as it is in the world. It is very important that the Van province has eligible meadows and pastures that contain wide flora characteristics in terms of honey yield. At the same time, the value of bee products produced in these areas is an opportunity for enterprises.

In this context, enterprises should be encouraged to produce main product as well as byproducts. As a result of the production diversity to be supported by the state, a high level of benefit will be provided from meadows and pastures eligible for beekeeping. Favoring of the laboratory analysis process within the scope of the recommended follow-up under the AKS is important in terms of tracking and recording the products which are produced here. Taking into account the high analysis fees, the analysis fees of trained competent beekeepers should be subsidized by the state and local governments.

Due to the problems experienced in the supply of queen bees, mainly hybrid bee breeds are used in enterprises. Efforts to creating the breed of queen bee specific to the region should be accelerated and we should be attentive to use of these queen bees by beekeepers. Regarding this subject, communication instruments and social media should be used effectively. Due to its nature, beekeeping is carried out in meadows and pastures at high altitudes in the untouched areas of Van. However, during accommodation of beekepeers, they are suffered from terrorism and face with security problems. In this case, specified accommodation locations within the province should be secured accordingly.

Bee products is a part of the smuggling activities performed in the region. Honey of unknown content, brought illegally across the border, is offered to the market at low prices. In this case, beekeeping in the region has been affected adversely. In addition to the unfair competition, this illegal honey market put public health in danger. In conversations held during the questionnaires with producers, they stated that they lost their bees after feeding them with food exported from Iran. In this context, it is critical to prevent illegal honey for the sector and public health. Prevention of terrorism and smuggling will have positive effects for beekeepers in the Van province.

As a result, strategies developed for positioning beekeeping in the region in a higher position will guide all parties. All strategies should be evaluated separately and all useful actions relevant to beekeeping should be taken.

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CHAPTER 6

SOIL ZINC (Zn) DEFICIENCY AND BREEDING IN PLANTS

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INTRODUCTION

The nutrients play a major role in physiological and biochemical functions of biological systems. While the high crops supply their nutrients from soil, the animals and humans supply mostly from high plants (Grusak and Cakmak, 2005). The mineral element deficiency, in particular, zinc (Zn) and iron (Fe) deficiencies affect more than 3 billions people all around the World (Welch and Graham, 2004). The zinc deficiency is the most common microelement deficiency in the World. The said element is essential for the humans as well as the plants. Because, it is catalytic component of over the 300 enzymes. The most important of these enzymes are alkaline phosphatase, alcohol dehydrogenase, Cu-Zn superoxide dismutase and carbonic anhydrase. In addition, the zinc plays a significant structural role in the proteins which regulates transcription such as Zn-Finger, Zn-Cluster and Ring-Finger and it is the only metal found in the six classes of enzymes (Barak and Helmke, 1993). The zinc plays a key role, in human, in physical growth and development, the functioning of the immune system, sexual health, sense of taste and nervous system (Hotz and Brown, 2004). In plants, the enzymes containing zinc or activated by zinc paricipate in carbonhydrate metabolisms, protein synthesis, cell membrane protection, regulation of the the oxine synthesis and pollen formation (Marschner, 1995). Cakmak (2000) reported that the zinc is essential for regulation of the working of genes in plants which show tolerance to abiotic stress conditions such as high light intensity and high temperature.

The physiological disorders caused by zinc deficiency appeared as dwarfing in plant, formation chlorosisin leaves, being small-leaved, shortening between nodes and shrinkage in the leaves asthey can not produce sufficient indole acetic acid. Besides, it causes yield and quality (protein content, largeness and complexion) casualties. The zinc deficiency also impairs the photosynthetic carbon metabolism. In consequence of the zinc deficiency, carbonic anhydrase enzyme containing zinc activity is decreased (Rengel, 1995). This enzyme catalyzes only one zinc atom and hydration of carbondioxide and it enables attachment of carbondioxide for cycle of Calvin-Benson (Marschner, 1995). Because the chloroplast structure is degeneratedand photosynthetic electron transfer is blocked in the severe zinc deficiency conditions, photosynthesis network is inhibited (Sharma et al., 1982). The zinc deficiency may lead to reduction between 50-70% in net photosynthesis level depending on the plant species and the severity of deficiency. The quality and yield casualties come up without the occurence of above symptoms in soils showing moderate zinc deficiency. This case is named as “hidden or invisible deficiency”. By starting the production of high-yielding new varieties, nitrogen-phosphorus-potassium (NPK) fertilizer amounts and the zinc deficiency which depends on high phosphorus

levels scaled up. Most of these varieties include "hidden zinc deficiency" and the yield and quality reductions prompt to significant economic losses (Alloway, 2009). In a study backed up by FAO and conducted between 1974-1982, it was reported that there is a zinc deficiency in 30% of the world's agricultural areas (Sillanpaa, 1982) and the zinc deficiency is seen in worked all territories except for areas outside Belgium and Malta.In addition to this, India, Pakistan, Iraq, Lebanon, Syria and Turkey's soils were reported to be the country having the lowest levels of zinc.

In one microelement project with which was conducted with 190 field tests in 15 contries by FAO, the zinc was detected as a microelement that the highest impact on yield (Sillanpaa, 1990). The results of project put forth that 51% of zinc deficiency is acute and 49% is hidden deficiency. Hidden zinc deficiency usually is related with the cultivation of varieties of zinc-inefficient in soils containing marginally lower zinc. Most studies show that zinc-efficient varieties can be grown quite successfully in the same soils (Graham and Rengel, 1993).

Zinc-efficiency or high zinc-efficient genotypes have a normal growth and yield capacity even in soil seen zinc deficiency enough to cause loss of yield and quality (Graham et al., 1992, Cakmak et al., 1996). It was reported that the high fitosiderof secretion of plants and physiological reasons, such as use of zinc more efficiently and better transport of zinc the necessary organs can take a place on the basis of zinc activity (Rengel, 1999). However, the genetic basis of this mechanism which specifies the zinc activity of the plant has not been well understood (Schachtman and Barker, 1999). This situation has hampered the "zinc efficiency" variety breeding.

The reason for obtaining higher yield from zinc-efficient varieties than non-zinc-efficient varieties in soils with zinc deficiency as follows:

1. Being efficient of the mineral uptake from soil causes to increase the plant tolerance to biotic and abiotic stress. Microelement deficiency makesthe plant vulnerableto against especially stem blight diseases (Graham and Webb, 1991). Zinc and phosphorus deficienciespresenting in the soil prompt that the sugars being activator for pathogenic agents, amides and amino acids leach out of roots. This situation makes the plant vulnerable to infections sinceplant roots blocked up by the pathogen rather than causing the microelement deficiency (Graham and Rovira, 1984; Sparrow and Graham, 1988; Thongbai et al., 1993). The variety breeding which has a high efficiency of microelement intake and usage may makeproof the plant to fungus diseases and so fungicide use is reduced.

2. Microelements-efficient varieties show a deeper root growth in soils having microelement deficiency and better advantage of water and nutrients in the soil. When the topsoil is dry, the rootsin this region lose their

activity. Plants survive their roots in deeper and continue to intake water and nutrients which will continue to yield potential. The presence of deep roots increases the plant's drought resistance as well (Graham and Welch, 1996).

3. The seed which is rich in microelements have higher germination power (vigor) and correspondingly it has a high yield.

Plant species and varieties show different tolerance to zinc deficiency. It was reported that there was a significant genotypic variation against zinc deficiency and its applications in many species such as Maize (Ozer, 1999; Ozguven and Katkat, 2001), Wheat (Torun, 1998; Hacisalihoglu et al., 2001), Summer Squash (Yagmur et al., 2002), Beans (Karaman et al., 1998), Chickpeas (Akay, 2005), Pepper (Gunes et al., 1999; Aktas et al., 2006) and among the varieties of these species.

One of the way to reduce zinc deficiency in plants is developing the zincefficiency varieties which will not show hidden zinc deficiency even if cultivated in the soil with low zinc, second solution which is temporal is that the fertilization program containing zinc should be applied (Cakmak, 2008). In the soils were seen element deficiency in the breeding in terms of nutrient uptake efficiency, certain genotypes are evaluated with regard to capability to intake this element and benefit from it. High yield and the breeding for more nutritious production purpose complete each other.

Any plant variety which has high Zn effectiveness hasn't been breeded until now. The first reason for this; zinc activity testing via selection is a slow process reuiring much labor, the latter; field tests show high environmental variance (slow genetic progress in phenotypic selection), the third; characters show complex structure conrolling multiple genes. Because the zinc activity shows complex structure as a genetic/physiological and being slow genetic progress which will be obtined from phenotypic selection in developing varieties having high zinc activity (low inheritance level), marker assisted selection (MAS) is acceped as a strong alternative.

Breeding for Zinc Effectiveness in Plants

One of the plant breeding's aim is increasing the production by developing plant having high yield. Yet, impacts of the microelements on the plants and enhancing nutrient content take a part among the breeding purposes. Some nutritional problems seen in plants are not sonved with chemical fertilization. For this reason, plant variety and species breeding to adopt soils having nutrient element deficiency is very important. Microelement deficiencies arising from high pH may not remedy with fertilization, thus, genetic solutions are necessary. In addition to this, as the hitches in the soil layers, drying subsoil and reasons resulting from diseases, fertilization

may not be effective for fulfilling a Zn need (Graham and Rengel, 1993). So, it is effective and sustainable solution for Zn deficiency restricting using and producing of Zn-effective genotypes in Zn-deficiency condition.

Zn content of seed could be increased with soil and foliar fertilizer. At the same time, Zn contents may be augmented by means of applications to seed (Riberio, 1993; Orioli et al., 2008). In addition to all alternatives, the cost depends on non-renewable sourcing. Besides, the genetic progression is seen as a cost-friendly and also the most influential strategy.

The scanning in order to determine genetic variations in the concentration of elements present in the tissues constitutes first step of breeding. Plant vary significantly in point of elements such as Zn. For instance, Zn concentration was determined as in the range of 14-42 mg Zn/kg and approximately 25 mg Zn/kg in the wheat seed. In a different study, when the Fe concentration varies between 8 and 24 mg/kg, Zn concentration varies between 14 and 42 mg/kg y analysing wheat genotypes in the terms of Fe and Zn concentration. Similar ratios were obtained in paddy in Fe and Zn concentration (Bouis, 1996).

The 4 approches were selected to characterize the tolerance to zinc deficiency in wheat and barley: (1) The relative weight of shoots by comparing high and low Zn conditions. (Zn activity index) (2) The relative weight of shoot/root ratio by comparing high and low Zn conditions. (3) Total zinc intake of shoots in Zn deficiency. (4) Dry shoot weight in deficiency conditions (Lombnaes and Singh, 2003). The consideration of Zn activity ratio together with severe deficiency symptoms can be reasonable approach in terms of scanning rapidly and fidingly a great number of populations for zinc activity (Cakmak and Braun, 2001; Genc et al., 2003).

For instance, through the selection to be done from the population which is segregated in *Phaseolus vulgaris* or controlled hybridization, it seem to be possible that the zinc content in the seed can be increased to 10% (Gelin et al., 2007).

Genotypic Variation in Zinc Activity

Variations are in existence in tolerance to Zn deficiency in terms of Zn intake and usage among the plant genotypes. The studies about tolerance to zinc deficiency was conducted in wheat, barley, rice, chickpea and maize and it was reported that genetic variance is enough for breeding in this subject (Khan et al., 1998; Genc et al., 1999; Banziger and Long, 2000; Beebe et al., 2000; Gregorio et al., 2000; Ortiz-Monasterio and Graham, 2000). Although some indications are acquired about genetic x environment interaction nutritional traits are stable among the environments. This situation shows that high yield with high nutritional traits can be combined (Gregorio, 2000).

The studies about morphologic and physiological plant factors was conducted to understand base of genotypic variation forming in Zn deficiency conditions. Studied factors to date are; (a) Severity of Zn deficiency symptoms in leaves (b) dry matter production of root and shoots (c) Emission of phytoryderophors moving from root by zinc (d) Yield (e) Transportation and concentration of zinc in plants (f) Zn intake from root and its transportation to shoots (g) Zn-superoxide dismutase (SOD) activation Cereals' reactions are quite different to Zn deficiency and its fertilization. When oat and durum wheat was rather vulnerable to Zn deficiency, triticosecale and espeially rice showed high Zn activity. The said species' susceptibility to Zn deficiency dependently severty, biomass production and abating in grain yield of Zn deficiency can be listed as follows: Durum wheat> Oat> Bread wheat> Barley> Triticale> Rye (Cakmak et al., 1997a; 1998; 1999).

It was reported that although the difference in Zn activity correlates with total Zn amount within and between Cereal species, it does not correlate with shoot dry matter amount. But, Zn-including superoxide dismutase activity which is found in Zn-active rye and bread wheat's leafage is higher than Zn-inactive rye and bread wheat species. This situation means that physiologically active Zn amount in leaf tissue of active genotypes is higher. The marked Zn (Zn_{62}) intake and its transportation to shoots in Zn-efficient rye and bread wheat genotypes is higher than the Zn-inefficient ones (Cakmak et al., 1998).

In a study on the purpose to determine Zn efficiency of 6 diploid, 9 tetraploid and 7 hexaploid wild and primitive wheat genotypes, severe zinc deficiency showed up quickly in whole tetraploid wheat genotypes. Diploid and hexaploid wheat genotypes are more vulnerable than tetraploid genotypes. Average Zn efficiency ratio was determined as 36% for tetraploid, 60% for diploid, 64% for hexaploid genotypes. Not only changes in Zn intake capacity of genotypes, but also role in the expression of inherent zinc utilization capacity were propounded by this study. Besides, the potential source of genes determining Zn activity of A and D genomes in wheat was enounced to be important (Cakmak et al., 1999).

Zn efficiency among the genotypes do not correlate with Zn concentration and scope of seed. It was determined that wild wheat seeds have more Zn content than cultured wheat seeds whereas wild tetraploid wheats (ssp. *dicoccoides*) having low Zn activity have the second highest seed concentration. In another study conducted similarly, although the Bezostaja (hexaploid wheat) has low Zn concentration in low Zn condition, it was determined to perform Zn activity at the highest rate. The results of study showed that Zn content in shoots show variation between varieties and genotypes. The genotypes which have high Zn activity had usually more

Zn content. There was no correlation between Zn activity and Zn content at high rates. But, it was reported that correlation between Zn activity and content of plants were higher rate than Zn concentration (Cakmak et al., 1999). In another conducted study with 37 bread wheat and 3 durum wheat in Zn-deficient field conditions and 21 bread wheat and 3 durum wheat in greenhouse conditions, Zn fertilization and grain yield showed variation range from 8% to 76% rates between varieties. Average zinc efficiency was between 57-92% in field test while it was varied between 47-83% in greenhouse test. A large part of varieties reacted similarly against Zn deficiency in field and greenhouse tests. The varieties selected from local area had both high yield and high Zn efficiency in Zn deficient conditions. Bread wheat species usually had low yield Zn efficiency and in both tests. On the other hand, all the durum wheats showed grain yield, shoot dry matter weight and Zn efficiency at the low level in Zn-efficient conditions. But, there was no correlation among grain Zn concentration, shoot dry matter weight and Zn efficiency (Kalayci et al., 1999).

Rengel and Wheal (1997) indicated that the differences in Z efficiency between wheat species was affinied with significant differences in zinc intake capacity. It was declared by various researches that there was a positive correlate between Zn activity and total Zn amount of shoots in field tests in zinc-deficient calcerous soils (Graham et al., 1992; Cakmak et al., 1997b). Although Zn-efficient genotypes have high Zn intake ability, some genotypes do not include high Z concentration in leaf, shoot tissue and grain (Graham et al., 1992). Moreover, Zn inefficiencient wheat genotypes can have higher Zn concentration than Zn efficient genotypes in grains and leaves (Rengel and Graham, 1996; 1997b; 1998).

Zn deficiency is one of the factors that reduce the rice yield. After understanding the present case, the variation was analysed in terms of Zn contents of leaves and seeds in paddy genotypes and although there was great huge variationn in Zn content of leaves, any correlations was not reported between leaf and seed Zn level (Nagarathna et al., 2010).

It was reported that Zn efficiency of 164 bread wheat genotypes varied between 33% and 77%. The tolerance to Zn deficiency was independent from Zn concentration but it was found as affinied with total Zn content which presented in every shoot. It was reported that dry shoot matter weight of Zn active genotypes was 1.6 fold higher than low Zn-active genoypes in Zn deficient condition (Torun et al., 2000).

In a study was conducted to determine the impact on the Zn effectiveness of different Zn application in 4 different maize species, Zn effectiveness varied between 63.5% and 81.2% in 40th day, 63.5% and 81.2% in 80th day of harvesting. Zn application caused a upsurge in dry matter

weight off all varieties. It was reported that Zn concentration of shoots correlated with Zn effectiveness (Chaab et al., 2010).

Rye (Secale cereale L. cv. Aslim), Triticosecale (*Triticosecale* Wittmark. cv. Presto), 2 bread wheat (*Triticum aestivum*. cvs. Bezostaja-1 and Atay-85) and 2 durum wheat (*Triticum durum* L. cvs. Kunduru-1149 and C-1252)'s responses to Zn deficiencies were indicated in Zn deficient calcerous soil and Zn-sufficient field and greenhouse tests. The first visual symptom of Zn deficiency was reduction in shoot growth following the browning in leaf blade. These symptoms were seen little of nothing in triticosecale and rye. But, especially in durum wheat, it showed up more rapidly and waxily. The decreasing in grain yield and dry matter weight resembled with symptoms. Zn effectiveness which was calculated in point of dry matter producction in cereals was indicated as 99% in rye, 74% in triticosecale 59% in bread wheat and 25% in durum wheat. The vulnerability to Zn amount in shoots, but it did not correlate with Zn concentration in the dry matter. When Zn was applicated, it was moved to shoots in high amount in the rye and triticale than wheat. These all results showed that Zn effectiveness was aligned as rye> triticale> bread wheat> durum wheat (Cakmak et al., 1997a).

75 pure pepper lines (15 sweet pepper, charleston, capia, long green pepper and ornamental pepper) were tested with zinc (5 mg kg^{-1}) and Zn-deficient (0 mg kg^{-1}) in greenhouse conditions. The Zn efficiency values of all pure lines tested with this study varied between 7.1% and 48.1%. These Zn efficiencies of pure lines were determined as 18.1%. Only sweet pepper had higher Zn efficiency than this average value. Respectively charleston (17.7%), oil (17.5%), long green pepper (16.7%), ornamental pepper (13.1%) followed to this (Eken, 2007).

766 *Triticum* speltas were tested for grain Zn, Fe and N concentration in different locations by Erdem (2009). Results showed that spelta wheat genotypes had the highest Zn and Fe concentration and huge varition in the sense of these element concentrations in grains. In addition, N concentration increased as long as Zn concentration increased in grain. It was determined that there were positive correlates between grain Zn-Fe concentration and Zn-N concentration in grains (r=6179***). Zn efficiencies of 72 genotypes varied between 43-95% (average 79%).

Heritabilty of Zinc Efficiency and Molecular Studies

The inheritance is measurement of phenotypic differences which is obtained depends on genetic differences in point of one trait. The inheritance can be expressed in two different ways: (a) In a broad sense (H^2), (b) In a narrow sense (h^2). Inheritance in a broad sense measure the phenotype's

ratio (V_P) which is occured by the reason of genetic variation (V_G) for a population in environmental limits during the experiment. Impendency of inheritance value to "1" shows that the environmental impact is a little to phenotypic variation obtained from a population. Being close to "0" shows that only the environment is responsible for almost all the differences. Inheritance in a narrow sense (h^2) propound ratio of phenotypic variation which occurs thanks to additive genetic variation. Inheritance in a narrow sense is beneficial during estimation of phenotypes of offsprings. If it is close to "1", it is possible to make a precise estimate of the phenotypes of offsprings based on the information of their parents phenotype. Inheritance in a narrow sense is calculated as h^2=VA/VP (Klug and Cummings, 2005).

Inheritance estimates show that inheritance in a broad sense is for Fe (H^2=0.73), for Zn (H^2=0.81) and for dry matter (H^2=0.93) between sweet potato genotypes. At the same time, there is positive correlate between Fe and Zn content of sweet potatoes' repository roots. The previous studies show that genetic variation is too little between roots of similar plants (Courtney, 2007). The main purpose in breeding of sweet potato is to develop tubers with high Zn and Fe contents. Obtained evidences showed that the breeding techniques can be used in obtainment varieties which have high microelement efficiency. Because potatoes have high genetic variation and heredity rate in point of microelement content.

Many scientific study about Zn efficiency are related to physiological aspects of Zn intake. To that end, the genotypes are compared between each other in terms of relative Zn efficiency depending on growth in soils which have low avalible Zn content. Although there is no report about physiologic mechanism in Zn efficiency, the informations are limited about genetic controls of these mechanism and identifying molecular base and genes being answer for Zn efficiency. The genetic differences for Zn efficiency and their heredities are able to enable cantinuation of the process on developing of Zn effeciency in plants.

A limited number of studies on several plant species put forth some evidences about genetic of Zn effeciency. The conducted studies with rye lines showed that Cu, Zn and Mn effeciencies are independent characters and they take part in different chromosomes (Graham, 1984). As well as in rice's diallel analysis results showed that genes controlling Zn deficiency are additive and higher dominant (Majumder et al., 1990). It was reported that the Zn concentration in spike leaves are controlled by 4 additive genes in maize (El-Bendary et al., 1993). Rye's Zn effeciencies are increased by several genes in chromosomes 1R and 7R. It was reported that the genes in armlet of chromosomes 1R and 7R are more effective (Cakmak et al., 1997c; Schlegel and Cakmak, 1997). The range of F3 population obtained by hybridization of Zn-effecient and Zn-ineffecient soybean genotypes

(Hartwing et al., 1991). The results which are obtained by F1 plants derived from diallel hybridization of zinc effectiveness different 7 wheat genotypes showed that genes controlling Zn effeciency are dominant (Cakmak and Braun, 2001).

ZNT1 gene which can be expressed and caused to increase in Zn intake are indicated by numerous studies in Thlapsi species (Lasat et al., 2000; Pence et al., 2000; Assuncao et al., 2001). ZNT1 gene is a member of ZIP familiy carrying one type of two covalent cation (Guerinot, 2000). Expression of Zn *Arabidopsis* carrying gene in barley causes to increase short term Zn intake and seed cation content (Ramesh et al., 2004).

At the same time, decrease in RNA level of *T. aestivum*'s apical meristem (TaNAM) correlate with increase in N, Zn and Fe residues in flag leaves and decrease in Zn and Fe concentration of wheat grains. It was reported that this situation originates from decreasing Zn transport in leaves rather than dilution effect caused big grains (Uauy, 2006).

Dihaploid population which is obtained in consequence of hybridization between Zn-efficient RAC875-2 and moderate efficient Coscades genotypes showed negative correlation with shoot biomass and shoot Fe-Zn concentration in low Zn conditions. In a similar study conducted on the purpose of determination QTL which is connected with Fe and Zn concentrations in leaves and grains, the significant genetic correlation was obtained between grain Fe and Zn concetrations. It was reported that shoot biomass and tissue-grain Zn concentration are controlled by a major gene and several minor genes. QTL was calculated for 12-81% genetic variation depends on trait. Most of QTLs were correlated with seedling growth and plant length in Zn deficiency conditions. 1 QTL was determined for Fe concentration, 4 QTLs for grain Zn concentration. One QTL correlating with shoot Zn concentration, grain weight and the severity of zinc deficiency was found in chromosome 4. Another QTL correlating with shoot and grain Fe concentration and grain weight was determined in 4D chromosome. These two loci promise hope for studies which will be conducted in the future about molecular marker development working to increase in growth depending on the iron and zinc intake in low Zn conditions. 4QTLs were determined associated with grain Zn concentration. TheseQTLs took part in 3D, 4B, 6B and 7A chromosomes and they explained 92% genetic variation. Each QTLs have a relatively small effect on grain Zn concentration. But, these 4 QTLs cause to increase in grain Zn content up to 23% when they come together. This situation refers to necessity of gene combination (Pyrimiding) in development of grain Zn content and determination of more effective QTLs for increasing in Zn concentration (Genc et al., 2009).

F1 and F2 generations obtained from resiprocal hybridization of 3 varieties and backcross populations were used in a study conducted on the purpose of estimation of Zn content in bean grains. Dry matter Zn contents were determined as 57.5% for middle and 77.84% for high in inheritance in a narrow sense and they varied between 21.76 and 53.48 mg/kg. At the same time, trangressive segregation was obtained. The results showed that Zn contents of bean seeds can be increase using with tried 3 parents. It was reported that they could ensure 37.3% raise in Zn content (Da Rosa et al., 2010).

Zn content in the small white beans (navy bean) are controlled by a dominant allel and additive gene (Cichy et al., 2005). Inheritance in a narrow sense was determined as 82% by a study conducting with Voyager and Albion's genotypes. This situation refered that this trait was controlled by one gene and it had high heredity level. But, Zn content showed general range and it was controlled multiple genes in improved central America genotypes. These results indicate that there are stil doubts about how many genes control to Zn content (Rose et al., 2010).

The trangressive segregation was obtained by using Perola x Guapo Brilhante hybrid combinations (*Phaseolus vulgaris L.*). Zn content in the dry matter was determined as 53.48 mg Zn kg in F2 population and this value was corresponded to 37.26% increasing in Zn content. Similar results was obtained in studies which was conducted by Gelin et al. (2007). Trangressive segregation in terms of bean's Zn content was determined as well as in Blair et al. (2009)'s studies. These results show that the Zn content of bean seeds can be increased by genetic studies.

Two loci which correlate with seed Zn concentraiton and content were identified in barley cultivated field and greenhouse conditions. At the same time, the distributions of the measured characteristics, traits showed that trangressive segregation. This distribution shows that subject trait is controlled by expected multiple genes (Sadeghzadeh et al., 2010).

150 diploid lines which were advanced from Clipper (low Zn carrier) and Sahara 3771 (high Zn carrier) species were tested for seed Zn concentration and content in field and glass greenhose conditions in order to develop molecular markers for increase of Zn transportation in seed. One dominant DNA polymorphism was specified by using MLFP (Microsattelite-anchored Fragment Polymorphism) techniques. The applicant MFLP marker was isolated from gel and it was reproduced, cloned, sequenced, converted into PCR-based marker. This marker takes part in 2H chromosome's armlet and it can be useful to develop production volume and the nutritional qualitiy of barley in low zinc conditions. But, high Zn content in seed can't supersede single-handedly Zn fertilization (Sadeghzadeh et al., 2010).

Genotypic variations for Zn content in wheat seed were reported by different researches (Raboy et al., 1984; Moraghan and Grafton, 1999; Gregorio et al., 2000; Mantovi et al., 2003; Uauy et al., 2006). Increasing the amount of zinc content in consumed products contributes to meet the people's need for zinc. One of the effective method is selection for obtainin Zn-intensive seed in breeding programs. But, the mineral-intensive seed development is stil conducted depending on classic plant breeding approches inspite of high and time consuming labor requirements (Cakmak, 2002; Poletti et al., 2004; Welch and Graham, 2004; Ghandilyan et al., 2006). The plants with commercial mineral-intensive seeds by using molecular markers may be the most effective approches (Zimmermann and Hurrell, 2002). The molecular markers were used in characterizing of genetic locus where the seed Zn content presents there in Arabidopsis (Vreugdenhil et al., 2004; Filatov et al., 2007) and Bean (Guzman-Maldonado et al., 2003). One QTL being effecient in increasing of seed Zn content was specified in armlet of 2H chromosome in barley (Lonergan, 2001).

Shi et al. (2008) used diploid population which was obtained from Hanxuan 10 and Lumai 14 wheat genotypes hybridization to determine QTL for wheat grain Zn concentration and content. On the purpose of breeding wheat genotypes with high grain Zn concentration by using molecular markers and understanding the genetic control of Zn concentration. Besides, they analysed the phosphorus (P) concentration and content in wheat grains in order to determine whether there was interaction between these two elements. 4 QTLs for zinc concentration, and 7 QTLs for Zn content were determinedin this study. These 4 and 7 QTLs were found in the same loci. It was reported that this situation can enable development Zn content and Zn concentration, together. On the other hand, 4 QTLs for phosphorus concentration and 6 QTLs for phosphorus content were determined. 2 QTLs for Zn concentration and 6 QTLs for P concentration took place in 4A and 4D chromosomes. 4 QTLs for Zn content and another QTLs determined for P content were located in 2D, 3A and 4A chromosomes. This event represents the positive correlation between grain Zn and P concentration. In addition, it showed that Zn and P concentration were conrolled multiple genes.

The bean breeders manifested that the sharpest symptom is the plant have high death rate in the study which was conducted to determine tolerance to Zn deficiency. And, 4 QTLs which are connected with plant death were identified and only one of them was located in the same loci with one of 4 QTLs correlated with leaf browning. The most important 2 QTLs for plant death were determined in chromosomes 2 and 12 and the variation was determined as 16.6% and 24.2%, respectively. Besides, The allels of tolerant parent "Jalmagna" reduced plant death at the rate of 24.2% and

16.2%. While the QTLs was exhibiting additive behavior, the epistatic interactions were important for leaf browning (Wissuwa et al., 2006).

168 F7 RIL populations were used in QTL mapping for Fe and Zn concentration in rough rice grains. In this study, 5 QTLs which associated significantly with Fe and Zn in chromosomes 1, 3, 5, 7 and 12 were specified in genome mapping by using 101 SSR and 9 gene-specific markers. Total 14 QTLs were identified for two traits. The QTLs for Fe mucked on chromosomes 7 and 12 with other specified QTLs for zinc. 10 nominee genes were found close to 12 of the 14 QTLs and other 6 nominee genes were found close to QTLs which exist on chromosomes 3, 5 and 7 (Anuradha et al., 2012).

A study was conducted to evaluate iron and zinc concentrations of the seeds and heredity of the contents in the white bean population grown in 3 different areas of Columbia (Mesoamerican gene Pool-Mesoamerican recombinant inbred line-RIL) and to determine the QTLs for each mineral. In this study, QTL correlated to Fe and Zn was determined in the b06 linkage group and it was reported that important locus may be present in connection with mineral intake-transportation. Other QTLS for mineral concentration and contents were in the linkage groups of b02, b03, b04, b07, b08 and b11. When compared with the locus determined in the previous studies, importance of the locus in b06 connection group became more prominent. Discovery of an important locus for seed Zn/Fe concentration would give chance to the development of new types with the use of genotypes having high mineral contents especially in Mesoamerican gene pool (Blair et al., 2010).

In a study conducted on beans (*Phaseolus vulgaris* L.) by Cichy et al. (2005), it was reported that the seed's Zn content was controlled by a single dominant gene. Also, Brown et al. (2011) conducted a study to explore the Zn content of potato, the difference between the genotypes having the lowest and highest Zn content was reported to be 50% (12 and 18 µg/g dry matter) and the heredity degree in the widest sense was reported to be 0.61. Courtney et al. (2008) reported that Zn content in sweet potato differentiated six (6) times among the genotypes and calculated the wide heredity degree of the genes controlling the Zn content as H^2=0,82. Da Rosa et al. (2010) showed by using the populations F1, F2, GM1P1 and GM1P2 that heredity degree in narrow sense of the genes controlling the Zn content of the bean seed varies between 57.5% and 77.84%. El-Bendary et al. (1993) reported that general combination ability of the lines haing high Zn content is better and the Zn level in the leaf is controlled by minimum four genes. Gelin et al. (2007) reported that there was a locus (gene) controlling the Zn accumulation in the bean seed.

Richard et al. (2011) studied the natural Zn tolerance and accumulation in *Arabidopsis thaliana*. This study revealed that heredity degree in wide sense of the root and sprout grow and Zn contents of the plants grown in different Zn concentrations varied between 0.36 and 0.91. This study also revealed for the first time that Zn was necessary for the lateral root development. It was reported that Zn tolerance level in the genotypes has no correlation with the Zn content in the root and sprout.

81 diallel hybrids were developed with the use of 9 genotypes in soybean and was reported that Zn intake was mostly under the control of added genes (Spehar, 1995). In the study conducted to determine the heredity of zinc and iron transfer in the seeds of recombitant inbred lines obtained by the crossbreeding white bean genotypes having high and low mineral contents (DOR364 x G19833), 26 QTL was determined for mineral x trials x method combinations. Most of the QTLs determined for Zn and Fe were in B11 linkage group and this could be explained up to 47.9% of the fenotypical variation. It was thus reported that this locus was useful for marker assisted selection. Other QTLs were determined in the linkage groups B3, B6, B7 and B9. QTL in B11 connection group became prominent for the seed's Zn concentration and gave the highest LOD value (2.42-8.31). Transgression expansion was reported to be the most important evidence for both low and high mineral movement (Blair et al., 2009). And in a study conducted on cabbage (*Brassica napus*) by Wu et al. (2008), too many low effect QTLs were discovered in the zinc solutions having different concentrations in respect to mineral intake of the leaves.

Guzman-Mandonado et al. (2003) detected 2 QTLs explaining 25% of the seed's Fe content in the population grown by cross breeding the wild x culture genotypes and reported that heredity was controlled by multiple genes. In the same study, 1 QTL was detected explaining 15% of the seed's Zn content. Similarly, Forster et al. (2002) and Cihcy et al. (2005) reported that Zn transfer in the seed was controlled by a single gene. In the mapping study conducted by Gelin et al. (2007) by using the population used before by Chicy et al. (2005), it was reported that there was 1 QTL explaining 17.8% of the seed's Zn concentration. This QTL was reported to be present in the range of AN034D, V104D and K126G, Bng1 which close to the similar marker.

It was reported that Fe and Zn concentration contributed to the positive alleles with the effect of added gene to the parent G19833 having high mineral content heredity. Additionally, it was stated that transgression expansion was low and additive and high mineral alleles dominantly came from G19833 genotype. All of these results showed that the seed's Fe and Zn content was controlled by multiple genes and similar genes affected the movement of both two elements (Blair et al., 2009).

With the use of the population developed by interbreeding, *Arobidobsis halleri* × *Arobidobsis lyrata petraea* linkage map was drawn and 3 basic QTL affecting Zn tolerance in *A. halleri* were mapped (Willems et al., 2007). This study was conducted to compare *A. helleri* × *A. l. petraea* connection map with *A. thaliana* genome series. For that purpose, genetic linkage map was developed with 81 *A. thaliana* markers 23 of which was known to be in metal haemostasis. In the *A. thaliana* physical map that allowed evaluation of all genes located in similar regions, QTL confidence intervals were determined for Zn tolerance. One of the most important findings of this study was the re-determination that one third of 23 genes playing a role in metal hemostasis were located in QTL region that was thought to be the most important candidate for Zn tolerance (Nancy et al., 2008).

Low Zn content in the soil and plants affects Zn intake depending on diet in the world. In a study conducted on *Brassica oleracea* L., it was attempted to show whether there was sufficient variation in the sprout's Zn concentration to develop species of leafed vegetables. Sampling was made in the *Brassica oleracea* L. gene pool containing so many materials (n=376), representing most of the allelic variation in the world and also containing 74 commercial species (mostly F1). Although there was difference in the sprout's Zn content in the genotypes and it was observed that environment made a big impact to the sprout's Zn content. It was determined that heredity of the sprout's Zn content was important but was also relatively low among 90 dihaploid (DH) lines comprising a mapping population. Although several QTL correlated to the sprout's Zn were discovered in chromosomes C2, C3, C5, C7 and C9, they were weak and affected by the frowing conditions. For that reason, it was reported that improvement for the Sprout's Zn level was difficult in *B. oleracea*. It was further reported that while the sprout's P concentration regularly increased in all genotypes in case of soil conditions with low Zn, the sprout's Zn of only one genotype increased in low soil P level (Broadley et al., 2010).

A study was conducted to show Zn and Fe accumulation potential in lentil (*Lens culinaris Medikus subsp. culinaris*). In this study, total Fe and Zn concentrations of 19 lentil genotypes grown in 8 different locations of Canada (Saskatchewan) for 2 years were analyzed. Total Fe and Zn concentrations varied between 73-90 mg Fe kg^{-1} and 44-54 mg Zn kg^{-1} among the tested lentil lines. The heredity in wide sense estimated for Fe and Zn concentration in the lentil seeds was calculated as 64% and 68%. The results of this study evidenced that lentil may be hopeful in remedying nutritional deficiency arising from Fe and Zn deficiency (Thavarajah etal., 2009).

Micro element deficiency named as concealed starvation affects many people in the world. Theoretically, supply of extra zinc with the foodstuffs

could be possible with the selection improvement methods. In a research conducted to determine the characteristics of mineral element accumulation in the relevant region of the barley genome in dihaploid barley (*Hordeum vulgare*) population, 9 locus displaying expansion in the population in the process starting with growing and ending in maturation were determined to be correlated to the measured characteristics. 5 of them contributed to the Zn content of the grain. 3 allele grain increased the Zn content and concentration up to 53% and 75% in average. These results produced information about the process of assisting the improvement programs organized to increase Zn content in the wheat (*Triticum aestivum*) known to be a major nutritional source with the use of marker assisted selection method (Lonergan et al., 2009).

In another study conducted by Peleg et al. (2009), transgression affect that was very important in terms of whole grain mineral concentration and wide genetic variation were achieved in 152 tetraploid wheat population obtained by breeding pasta wheat (Langdon type) and wild dark wheat (*Triticum dicoccum*-(G18-16) species. Total 82 QTLs was mapped for 10 minerals. Important positive correlations were determined between the grain's protein concentration, Zn, Fe and Cu and QTLs detected for these characteristics. These determined QTLs may give a chance for the development of nutritional quality of elite wheat genotypes with the use of wild alleles especially for the purpose of enhancing protein, Zn and Fe contents. Total 6 of them were determined explaining 1-23% of the correlation with the grain's Zn concentration. High Zn content was seen in 5 loci (2A, 5A, 6B, 7A and 7B) of alleles belonging to the G18-16 genotype and in 1 locus (2A) of allele belonging to LDN (culture wheat) genotype. 3 QTL exhibited considerable genetics x environment correlation and while 2 of them (5A, 6B) exhibited similar characteristics in different environments, 1 of them (7B) was only found in one environmental condition.

And in a study conducted with the use of visual Zn deficiency symptoms, tolerance to the Zn deficiency in white bean was reported to be controlled only by one dominant gene (Singh and Westermann, 2002). In another study conducted to determine the heredity of Zn activity with the use of visual deficiency symptoms in barley, it was reported that tolerance to Zn deficiency at the seedling phase of the plant was controlled by a single gene which was not dominant (Genc et al., 2003).

Amoros et al. (2020) conducted a study to determine the broad-sense heritability (H2) and genetic gains achieved for iron and zinc concentrations in potato tubers and their relationships with yield components through three cycles of recurrent selection at the diploid level using sixty genotypes. They determined high phenotypic and genotypic coefficients of variation along with high H2 (0.81 ± 0.19 for both iron and zinc) suggested

that these parameters were under the control of additive gene effects and could be effectively manipulated by recurrent selection. As their results, iron concentration had the greatest positive direct effect on total number of tubers per plant, and zinc had a weak negative direct effect on average tuber weight. They stated that selected iron- and zinc-dense genotypes with high, positive general combining ability were identified for use in an interploidy (4x–2x) breeding scheme aimed at increasing the iron and zinc contents of stable, high-yielding disease and stress-resistant varieties.

Development of Molecular Markers for Zinc Efficiency

Zn deficiency responses in plants are controlled by multiple layers of regulation, including transcriptional regulation mediated by transcription factors such as F-group bZIP proteins, epigenetic regulation at the chromatin level, and post-transcriptional regulation mediated by small RNAs and alternative splicing. Understanding the regulatory mechanisms for zinc deficiency in plants will be important for improving Zn deficiency tolerance, Zn utilization efficiency and Zn biological enrichment in plants (Zeng et al., 2021).

For 20 years, molecular methods are widely used in the isolation, mapping and definition of the genes in plant species. Molecular markers provide ease in the creation of detailed physical and genetic maps in the plant species. Another important area of use for molecular markers is indirect selection where molecular markers related to the quantitative trait locus (QTL) are used in order to enhance the efficiency of classical plant improvement. Molecular marker practices help us to understand the physiological parameters controlling the plant's reaction to biotic and abiotic stress. Molecular markers have any advantages like not being influenced from the environment and providing information about all phases of the plant development.

Molecular markers could be generally classified in 3 groups. These are markers that could withstand hybridization (such as RFLPs), markers based on DNA chip and sequencing (such as SNPs) and markers that could withstand PCR (such as RAPD, SSR, STS, AFLP, MFLP and SRAP) (Sadeghzadeh, 2008).

Difficulties are experienced in the selection of important agronomic characteristics in the conventional plant improvement and these characteristics are influenced from the environment. Moreover, test methods are difficult and unreliable and they are also very expensive due to the nature of the target characteristics including abitoic stresses or due to the target environment. For example, microelement effcient lines are desired to be selected from expansion populations but it is very difficult to do that in

field conditions, in greenhouse or in growing rooms because the variation detected in the expression of these characteristics is considerably affected from certain factors such as the seed's nutritional content, detection of the characteristic, temperature and humidity content of the soil (Webb and Loneragan, 1988; Longnecker et al., 1991; Genc et al., 2000; Khabaz-Saberi et al., 2000).

It is impossible to define the micro-nutrient efficient genotypes in the population derived with the breeding of early generations because each plant type represents a different genotype. However, each genotype should be tested under low and sufficient micro-elemental conditions in order to show the genetic variation arising from the expansion of independent genes that are effective in the nutritional efficiency. For that reason, selection made with the molecular markers in especially improper conditions is considered as the most efficient improvement tool (Cakmak and Braun, 2001).

In the white bean, one QTL explaining 15% of the variation in the white bean grain's Zn content and included in the connection group IV which is related to the Zn concentration was determined (Guzman-Maldonado et al., 2003). And in barley, it was determined that existence of Sahara allele in Xbad175 locus of the short arm of 2H chromosome contributed to an increase of 20% in Zn concentration and 26% in Zn content. It was further determined that WG464 marker present in the long arm of 4H chromosome is correlated to the highly vegetative Zn concentration in anthesis phase. Marker analyses for the Zn concentration and accumulation in the sprouting showed that both of these characteristics were correlated to the two genes in the long arm of 4H chromosome (Lonergan, 2001).

In the forthcoming years, we are expecting to see important developments in the use of molecular markers in order to determine the gene or genes influencing Zn efficiency. These markers will be able to provide a chance for making selection aimed at Zn effeciency independently from the environment in various growing phases of the plants. If the markers are sufficiently close to the gene, they may be directly used in marker assisted selection. However, if the marker used for selection is far from the desired gene, problems arise due to the crossover occurring between the marker and gene. This results in erroneous conclusions in the screening phase (Mohan et al., 1997). For that reason, marker intensity and accuracy must be increased on the existing unsaturated genetic maps; genes must be detected and additional markers must be determined for MAS use. Markers based on PCR such as ALFP, SSR and MLFP may be used to enhance map saturation level and number of locus on the map (Karakousis et al., 2003). Genomic position of high Zn accumulation locus may be determined more accurately as compared to the existing genetic maps if saturated maps are used more.

In a study conducted by Pinar (2021) for the purpose of developing the related molecular markers, heredity of the genes controlling the Zn deficiency symptom in F2, BC1 and BC2 populations derived from the hybrid of *C. annuum* L. (Alata 21A) X *C. frutescens* L. (PI 281420) was determined and QTLs related to genes influencing Zn activity in F2:F3 population were detected. According to the results obtained from this study, a map was developed in the length of 926,6cM which contained total 62 polymorphic markers (31 SRAP, 19 SSR and 11 RAPD) and 12 connection groups. With the use of MapQTL.6 program, total 41 QTLS belonging to 9 traits related to Zn activity in F2:F3 population was determined. It was reported that findings could be used to develop pepper species having high Zn activity.

In Mexico one hundred and ninety recombinant inbred lines (RILs) which developed from 'Kachu' × 'Zinc-Shakti' crossing to mapping grain Fe and Zn concentrations also some phenologically and agronomically significant traits during the 2017–2018, 2018–2019 plant growing seasons Diversity Arrays Technology (DArT) and seven new pleiotropic quantitative trait loci (QTL) were identified for Zn and Fe grain on chromosomes 1B, 1D, 2B, 6A and 7D, controlling grain micronutrients and agronomic traits. The identified pleiotropic QTLs have expanded the variety of QTLs that can be used in breeding for the bioenrichment of wheat (Radman et al., 2021).

Major plants having Zn-intense seeds may be attractive for the farmers as they yield high income depending on high efficiency rates in soils having low zinc content. Studies conducted in the field and in the growing rooms showed a considerable genetic variation for Zn activity in barley. However certain inconsistent results were obtained in the studies conducted to determine the heredity of the variation and in the screenings made on the field and in the growing room. Use of molecular markers to support Zn activity can be considered as an important tool for the success of pepper improvement programs because of the difficulties in the development of fast and reliable methods to ensure activity or problems in accurately reflecting the results obtained in the field screenings. Determination of DNA markers for the detection of Zn activity may accelerate the development of those species that could continue to be efficient in soils having low Zn content and may serve as a beginning point for the definition of specific genes that are responsible for the differences in reaction of the plants to the Zn deficiency.

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CHAPTER 7

UTILIZING MACROALGAE PRODUCTS TO MITIGATE STRESS IN AQUACULTURE

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Introduction:

Food systems have generally prioritized terrestrial foods until recently. However, over time there has been an increasing focus on aquatic foods due to their unique role in providing food that is consumed globally (Gabriel and Akinrotimi, 2011; FAO, 2022a). Aquatic products provide important sources of essential fatty acids, micronutrients such as iron, zinc, calcium, iodine as well as vitamins A, B12 and D (FAO,2022a).

Apart from these, aquatic products have very important contributions to food security. For example, it meets 7% of the total protein in the world and 17% of animal protein (Gabriel and Akinrotimi, 2011; FAO,2022b). In addition, in some countries, the rate of meeting animal protein from fisheries reaches 50% in countries such as Indonesia, Cambodia, Bangladesh and Mozambique (FAO,2022b).

Over time, the contributions made to total aquatic products through fishing and aquaculture have changed in favor of aquaculture. Time-dependent developments in fishery products obtained through fishing and hunting are shown in Table 1 in terms of production and ratio.

Table 1 World Fisheries and Aquaculture production (Million tonnes live weight equivalent, except algae). (Adapted FAO, 2022a)

	1990	**2000**	**2010**	**2018**	**2019**	**2020**
Capture	88.9	90.9	91	96.5	92.2	90.3
Aquaculture	21.8	43.4	71.5	82.5	85.2	87.5
Total	110.7	134.3	162.5	179	177.4	177.8
World Fisheries and Aquaculture production rate (%)						
Capture	80.31	67.68	56	53.91	51.97	50.79
Aquaculture	19.69	32.32	44	46.09	48.03	49.21
Total	100	100	100	100	100	100

It is predicted that the increase in world fisheries and aquaculture production will continue for the next 10 years. According to the calculations, most of the production, which is expected to exceed the 100 million tons threshold for the first time in 2027, will come from the aquaculture sector. In 2030, this figure is expected to increase to 106 million tons. With these results, the share of aquaculture in total production is expected to increase from 49% in 2020 to 53% in 2030 (FAO, 2022b). In addition, while 56% of the fishery products that can be used for human consumption come from aquaculture in 2020, this rate is expected to reach 59% in 2030.

On the other hand, Increasing global demand for seafood has resulted in commercial aquaculture production being carried out in high stock densities (Hanke et al., 2020). This is because aquaculture practices around the world adopt intensive production models that will increase maximum productivity using available resources (Negm et al., 2021). However, this growth has come with the emergence of a variety of environmental and livestock-related stressors that are posing great challenges to the aquaculture sector and limiting its further expansion around the World (Hanke et al., 2020; Negm et al., 2021). Another important factor causing stress in aquatic organisms is global warming (Alfonso et al., 2021; Islam et al., 2021). All these stressful conditions and process in these intensive aquaculture environments have led to the deterioration of the health of the cultured animals, reduced resistance to diseases, and reduced growth performance, resulting in great economic losses (Gabriel & Akinrotimi, 2011; Hanke et al., 2020; Ciji & Akhtar, 2021) (Figure 1).

There are several challenges that need to be addressed, such as reducing the effects of stress, in order to sustain the increases seen so far and expected in the future in aquaculture. Therefore, stress management or reducing the effects of stress factors in aquaculture is an indispensable part of sustainable aquaculture development. While probiotics, prebiotics, organic acids and various plant extracts are used to mitigate stress in aquaculture, there has been an increase in the use of algae in recent years. In this review, the benefits of various macroalgae products for stress alleviation are discussed.

Stress and Stress Factors in Aquaculture:

Various different definitions of stress have been defined. Schreck and Tort (2016) defined stress as the general physiological response of fish to threatening situations, as in all other vertebrates. However, Raman et al. (2018) defined stress as "any biotic and abiotic factor that causes significant disturbances in an animal's normal functions, thereby reducing its probability of survival". In addition stress was defined by Francis-Floyd (1991) as a situation in which an animal cannot maintain its normal physiological state due to various factors and negatively affects its welfare. An inevitable part of intensive aquaculture is the manipulation of fish, which includes stocking, sorting, confinement, transportation and other farm operations from hatchery to final consumer (Gabriel & Akinrotimi, 2011). Stress can be short term and acute or long term and chronic and can affect various aspects of fish physiology and behavior (Toni et al., 2017; Ciji and Akhtar, 2021). While acute stress responses generally do not cause immunodeficiency in fish and other aquatic organisms, chronic stress emerges as a major problem in aquaculture, associated with poor growth, reproduction

and health of farmed all aquatic organisms (Mateus et al., 2017; Petitjean et al., 2019).

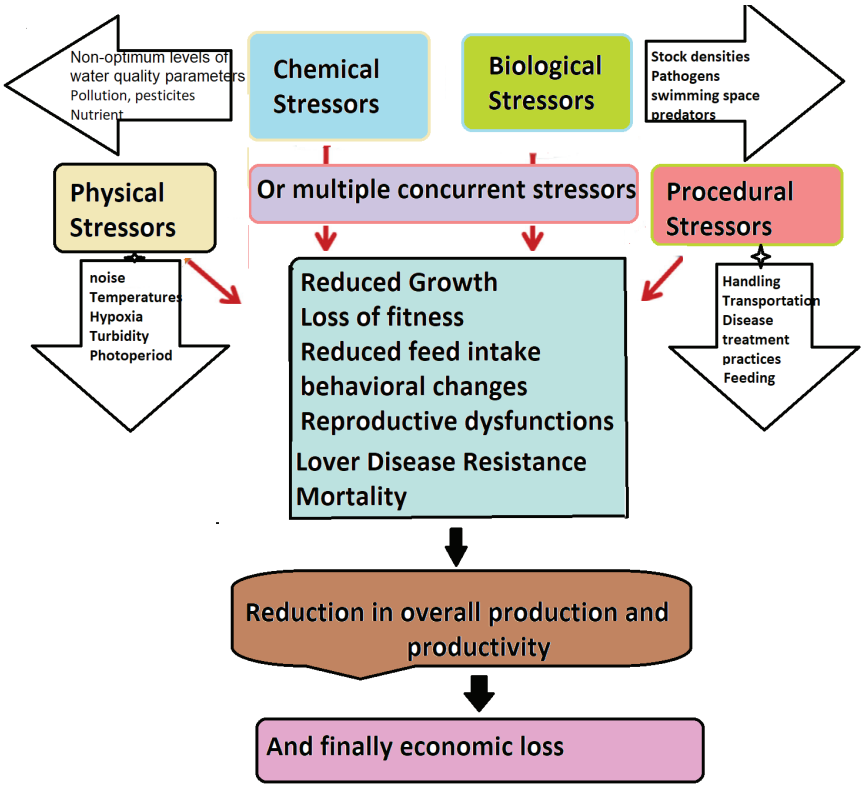

Figure 1. The overall effect of stress factors in aquaculture (Adaptated from Gabriel and Akinrotimi 2011and Ciji and Akhtar, 2021).

Stress response of fish

When aquatic organisms are exposed to stress, they show various reactions and exhibit physiological behaviors that will minimize the effect of stress. Although the reactions of stress factors may vary depending on some factors such as species, age, gender, maturity, type, size and duration of the stress factor, they generally exhibit similar reactions (Petitjean et al., 2019; Ciji & Akhtar, 2021). Adaptation of fish to stressors is highly dependent on the severity of the stressor and the duration of exposure (Barton, 2002; Petitjean et al., 2019). All of the stressors may elicit nonspecific responses that are considered to be adaptive so that the fish can cope with them and maintain their homeostatic state (Barton, 2002). If the stressor is too severe or too long for the fish to restore homeostasis, the responses may become maladaptive and threaten the health and well-being of the

fish (Barton, 2002; Islam et al., 2021). Fish exhibit an integrated stress response where behavioral, neural, hormonal and physiological factors come together to provide the best possible chance of survival (Gabriel and Akintromi, 2011). Physiological responses of fish to environmental stressors are grouped as primary, secondary and tertiary responses (Wendelaar Bonga, 1997; Petitjean et al., 2019). (Figure 2).

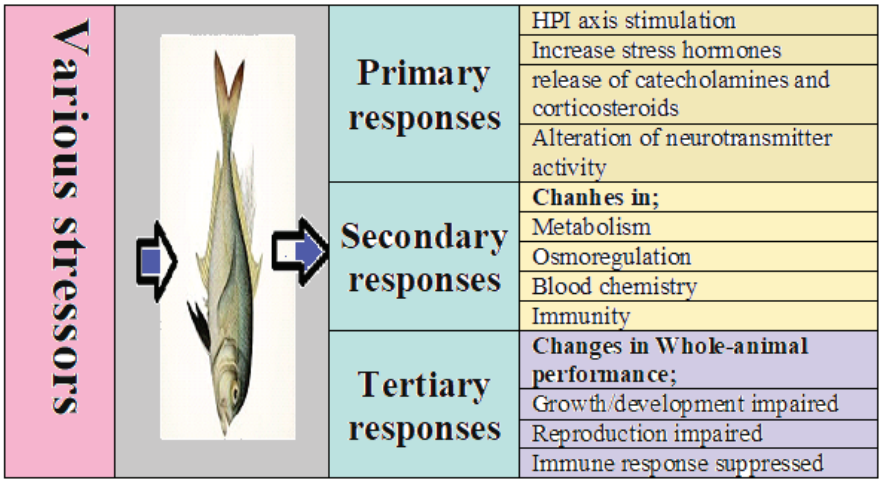

Figure 2. Stress response in fish

The primary response is the neuroendocrine response, characterized by secretion of catecholamines massively and induction of the hypothalamus-pituitary-interrenal (HPI) axis, leading to the release of corticosteroids into the bloodstream (Gabriel and Akinrotimi, 2011; Petitjean et al., 2019). Cortisol levels especially in plasma samples have been used as a primary indicator of fish well-being, particularly for acute stress (Mateus et al., 2017). Cortisol undertakes two main tasks such as regulating energy metabolism and maintaining mineral balance in fish (Wendelaar Bonga, 1997). The secondary response is usually characterized by changes in metabolism, osmoregulation, molecular chaperons or HSPs, blood chemistry, and immunity. These secondary responses are often adaptive at the cellular level (Schreck & Tort, 2016; Petitjean et al., 2019). In this context, plasma glucose concentration is the most commonly measured secondary response parameter to stress factors in fish, which is often used as an indicator of the stressful situation in fish (Barton et al., 2002; Ciji & Akhter, 2021). However, prolonged stress elicits tertiary responses that result in various pathological conditions affecting overall animal ans population performance, poor reproductive performance, overall resistance to disease, growth retardation and ultimately higher mortality (Magnoni et al., 2019; Magnoni et al., 2023).

Methods used to reduce stress in aquaculture

Although the emergence of stressors in aquaculture is inevitable, stress management in aquaculture is one of the main goals of producers or entrepreneurs in order to prevent negative effects on sustainable production and to optimize productivity (Gabriel & Akinrotimi, 2011; Kumar et al., 2015; Ciji & Akhter, 2021). Therefore, it has led to an increase in research on mitigating or eliminating the negative effects of stress in aquaculture (Varghese et al., 2020). It is suggested that studies aimed at reducing stress in aquaculture should be carried out in two main areas such as the management of the culture environment and the management of the cultured species. A healthy aquatic environment is one of the biggest concerns facing aquaculture facilities (Kumar et al., 2015; Varghese et al., 2020; Ciji & Akhter, 2021). While the management of the culture environment includes the management of water quality, general environmental management such as temperature, dissolved oxygen, pH, ammonia, nitrite and salinity, the management of the cultured species includes interventions to reduce the effects of stress in aquaculture conditions (Petitjean et al., 2019). These interventions include the use of anesthetics to reduce human-induced stressors such as grading, handling, transportation and netting and the use of different feed additives or nutraceuticals (dietary interventions) (Mateus et al., 2017; Magnoni et al., 2019; Ciji & Akhtar, 2021).

Stress management using functional additives in aquaculture can be an ideal strategy, since several studies have proven that various nutraceuticals have the potential to reduce stress in fish. Studies have shown that these dietary supplements have beneficial effects on well-being as nutritional modulators of metabolic pathways and immune systems (Magnoni et al., 2019). Morever, it has been suggested that unlike antibiotics, they do not cause any negative effects on the environment. Nutritive and non-nutritive compounds have been used in stress reduction through dietary interventions (Varghese et al., 2020). The most widely used nutritional compounds are Protein and amino acids, Essential fatty acids, phospholipids, carotenoids, vitamins, minerals and nucleotides. As for non-nutritive compounds, studies have been carried out to reduce stress with a wider variety of products such as Synthetic chemicals (Levamisole, Organic acids, Propylene glycol, Clay additives) and biological derivatives (Ciji & Akhter, 2021). As biological derivatives of non-nutritive compounds, prebiotics, probiotics, synbiotics, bacterial derivatives, polysaccharides, animal and vegetable extracts and macro and micro algae constitute the important part (Gabriel & Akinrotimi, 2011; Kumar et al., 2015; Ciji & Akhter, 2021). In this review, the stress-reducing effects of macroalgae and its derivatives on fish and other aquatic organisms against major stress factors such as low oxygen, ammonia, temperature and crowding are emphasized.

The use of macroalgae and its derivatives in stress reduction in aquaculture

Macro algae are considered in fish nutrition due to their essential amino acid content and high protein values, trace metals and vitamins. Additionally, macroalgae or seaweed can potentially be a low-cost protein source for fish (Saleh, 2020; Sayin et al., 2022; Yazici et al., 2022).

Considering data from previous studies, the use of macroalgae in fish diets can improve growth performance, feed efficiency, carcass quality, physiological activity, disease resistance (Hoseinifar et al., 2022). Morever, macro and microalgae and their extracts have been shown to increase stress tolerance in several aquaculture species to counteract adverse effects caused by various toxic substances or environmental stress factors. In challenge experiments, it was found that the survival rate of fish fed diets containing seaweed or their extracts increased by an average of 33% (Thepot et al., 2021).

Use of macroalgae to reduce the effects of hypoxia-induced stress:

In recent years, natural and anthropogenic degradation, including high temperature, algal blooms, water pollution, and high-intensity aquaculture, has caused local hypoxia in many parts of the world, severely restricting the natural distribution of aquatic species and aquaculture development. (Moyson et al., 2015; Shi et al., 2020).

In modern aquaculture production systems, where fish are dense and crowded, one of the vitally important water quality parameters that require constant monitoring to prevent disease outbreaks is the dissolved oxygen (DO) level (Abdel-Tawwab et al., 2019). Water quality often affects the growth, behavior, nutrient consumption and well-being of fish. In addition, hypoxia has significant effects on the physiological and immune responses of fish, making them more susceptible to diseases (Ciji & Akhtar, 2021; Zarantoniello et al., 2021). Therefore, the success of a commercial aquaculture production depends on ensuring optimum water quality with minimal resource costs for rapid fish growth and welfare (Li et al., 2018).

Hypoxia is a critical issue in aquaculture, especially in intensive aquaculture systems (Li et al., 2018; Varghese et al., 2021). Hypoxia, one of the known stress factors in cultured fish species, is a common event and fish respond with a complex series of physiological and biochemical, molecular and behavioral changes to cope with this environmental stress (Abdel-Tawwab et al., 2019; Shi et al., 2020).

These responses may differ from species to species (Varghese et al., 2021). However, in fish, O_2 uptake can be increased by changes in the morphological structure of the gills. Also, hematocrit and/or hemoglobin binding affinity may increase to maintain oxygen uptake and distribution

to tissues (Moyson et al., 2015). In fish grown at high stock densities, the antioxidant system is damaged due to insufficient dissolved oxygen level, and an increase in reactive O_2 species (ROS) is observed in tissues (Magnoni et al., 2017; Schleder et al., 2017; Shi et al., 2020). Moreover, it causes structural damage to the gills and skin, increasing its permeability to water and ions, thus leading to systemic hydromineral disturbances (Wendelaar Bonga, 1997).

Fish exposed to acute and chronic stress have different adaptive mechanisms. It was revealed that fish can use carbohydrate as the main energy source during acute hypoxia stress and metabolize more lipids during prolonged hypoxia stress (Li et al., 2018). In teleosts, hypoxia elicits a rapid release of hormones, including catecholamines and cortisol, followed by an organismal adaptive stress response, which consists of a series of secondary responses including high blood sugar and lactate content (Xiao, 2015; Moyson et al., 2015).

Some studies have been carried out to increase the resistance of macroalgae to low oxygen levels of different aquatic organisms (Magnoni et al., 2017; Shi et al., 2019; Salem et al., 2021). Shi et al. (2019) exposed the fish to ammonia for 24 hours after 8 weeks of feeding with juvenile black sea fish (*Acanthopagrus schlegelii*). An increase in cortisol level and oxidative stress was observed in fish, and humoral immunity was suppressed. However, it has been suggested that adding 6% of *Sargassum horneri* to the diet reduces cortisol and glucose, increases antioxidant enzyme activities, and improves immune response, so it can be used to alleviate the effects of stress caused by hypoxia (Shi et al., 2019).

Macroalgae contain biologically active substances with effective antioxidant capacities (Hoseinifar et al., 2022; Sayin et al., 2022; Yazıcı et al., 2022). Magnoni et al. (2017) reported that sea bream (*Sparus aurata*) exposed to acute hypoxia increased their tolerance for acute hypoxia when fed diets supplemented with heat-treated *Gracilaria vermiculophylla* and *Ulva lactuca* macroalgae (5%). It has been shown that by downregulating the gene expression of different antioxidant enzymes and molecular chaperones during recovery, it reduced the oxidative stress response in the liver and heart, reducing the need for antioxidant enzymes (Magnoni et al., 2017). Salem et al. (2021) stated that the addition of 4.1 g / kg *Laurencia obtusa* to the diet improves the growth performance and feed utilization of red tilapia (*Oreochromis niloticus* x *O. mossambicus*), and increases the ability of the fish to withstand stress.

All these studies have shown that the antioxidant properties of macroalgae may contribute to resistance to stresses caused by hypoxia (Magnoni et al., 2017; Salem et al., 2021).

Use of macroalgae to reduce the effects of ammonia-induced stress:

Ammonia, especially in intensive culture systems, is one of the most important water parameters after oxygen, which affects the behavior and health of fish (Negm et al., 2021; Zarantoniello et al., 2021). Ammonia, an end product of protein catabolism and constituting more than half of the nitrogenous waste released by fish, is one of the main environmental pollutants that affect the survival and growth of organisms (Cheng et al., 2015; Divya et al., 2020). It has been reported to increase directly in intensive farming systems (Israeli-Weinstein & Kimmel, 1998; Herrera, 2015). It may also enter water bodies as a result of the decomposition of sewage wastes, industrial wastes, agricultural wastes and biological wastes (Shi et al., 2019).

In the aquatic environment, ammonia exists in two different forms, relatively non-toxic, ionized ammonia (NH4) and non-ionized, uncharged, toxic ammonia (NH3) (Zhao et al., 2020). NH3 can diffuse easily across gill membranes due to its lipid solubility and lack of charge, while NH4+ occurs as a larger hydrated form with charged entities that cannot easily pass through hydrophobic micropores in the gill membrane (Cheng et al., 2015). Further, it is to be noted that the unionized ammonia molecules (NH 3) are toxic to fish at the level of 0.1 ppm while the ammonium nitrogen (NH 4+ N) at 1.0 ppm (Raman et al., 2018).

Ammonia poses a biochemical and physiological hazard to living organisms, especially in intensive culture systems with high stocking density (Zhao et al., 2020; Negm et al., 2021). It is suggested that ammonia can cause decreased growth, tissue erosion and degeneration, especially in the gills, suppression of immunity and high mortality in aquatic organisms, (Cheng et al., 2015; Zhao et al., 2020). Ammonia can also damage important biomolecules such as DNA, proteins, and lipids in organisms by increasing the concentration of reactive oxygen species (ROS), and may initiate a series of events, leading to disruption of cellular function (Cheng et al., 2015). In addition, it has been reported that increased ammonia levels show behavioral changes in fish, approaching the surface and becoming insensitive to feed (Israeli-Weinstei 1998; Jian-Yu et al., 2005; Shi et al., 2019).

Ammonia is not only harmful to aquatic organisms in high concentrations. For example, fish exposed to low levels of ammonia over time are more susceptible to bacterial infections, have poor growth and do not tolerate routine handling. It has been reported that when carp (*Cyprinus carpio*) is exposed to low concentrations of ammonia (0.033 mg/l) for 5 weeks, stress symptoms occur in the immune system and changes in blood

and hemopoietic tissue, head kidney, and spleen structure. An increase in glucose levels was also observed (Israeli-Weinstein & Kimmel, 1998).

In a study, it was reported that ammonia stress caused oxidative stress and an increase in cortisol levels in fish exposed to ammonia for 24 hours after 8 weeks of experimental feeding. In addition, the humoral immunity is suppressed during the stress period. However, juvenile black sea bream fed with *S. horneri* showed significant improvements in immune response, antioxidant capacity and resistance to ammonia stress, especially in the 6% *S. horneri* group. Moreover, ammonia stress can reduce immunity and cause oxidative stress in the liver, while the effect of ammonia is mitigated by supplementation of *S. horneri* in the diet (Shi et al., 2019).

Chen et al. (2012) showed that the immune parameters of white shrimp (*Litopenaeus vannamei*) fed with a diet supplemented with extract of *Gracilaria tenuistipitata* from the red algae returned to their original values in 12-24 hours after exposure to 5mg/L ammonia stress, while the immune parameters of the control shrimp returned to their original values in 72–120 hours. In another study, Suwaree et al. (2015) suggested that white shrimp placed in fucoidan-containing seawater exhibited the ability to maintain homeostasis by regulating cellular and humoral immunity to ammonia stress.

Use of macroalgae to reduce the effects of temperature stress:

Climate change poses a serious environmental risk factor for disease outbreaks in fish farms due to rapidly deteriorating water quality parameters (Islam et al., 2021; Maulu et al., 2021; Varghese et al., 2021).

Fish are ectothermic organisms that cannot regulate their body temperature and their physiological state depends on the ambient temperature. Abrupt changes in environmental temperature directly affect the immune systems, antioxidant defenses and survival rates of fish (Stehfest et al., 2017; Mariana et al., 2023). Therefore, temperature is one of the most important factors affecting poikilothermic organisms (Kumar et al., 2015; Urbinati et al., 2020). In addition, temperature is an important factor influencing their biological geographic distribution, as daily and seasonal temperature changes have an impact on the lifetime of individual fish (Nakano et al., 2014; Rehman et al., 2017). Morever, increases in temperature result in lower water oxygen concentration, which means higher reproduction rates and shorter incubation times for pathogens, as well as placing fish more stressed (Barber et al., 2016; Alfonso et al., 2021).

Acute temperature fluctuations cause oxidative stress as well as neuroendocrine, physiological, metabolic, osmoregulatory, and immunity impairments in fish (Alfonso et al., 2021; Islam et al., 2021). Fish elicit gen-

eralized physiological and immunological stress responses to heat stress as well as other stress responses (Rehman et al., 2017; Mariana et al., 2023).

While it is crucial to find ways to improve the physiological fitness of fish where water temperatures fluctuate greatly, there are currently few strategies available to reduce their adverse effects on fish (Islam, 2021; Varghese et al., 2021). Temperature control is possible in highly controlled aquaculture systems. However, in large closed waters such as lakes, ponds and open water cage culture systems, it is not possible to control the water temperature. Therefore, better alternatives are required for fish raised in such places (Ciji & Akhter., 2021; Islam et al., 2021).

It has been suggested that nutritional management strategies using functional and complementary feed additives may be another promising option to ameliorate thermal stress in fish, which we will face especially in the future (Kumar et al., 2015; Sun et al., 2021). For example, essential oils, or dietary selenium nanoparticles, zinc nanoparticles, mushrooms, fennel seeds, carotenoids, and vegetable oil have been reported to reduce thermal stress (both low and high) in different fish (Kumar et al., 2015; Ciji et al., 2021; Islam et al., 2021). However, the effectiveness of dietary and nutritional manipulation depends on the species, types of ingredients, concentration, dosages, duration and route of administration (Schleder et al., 2017; Hoseinifar et al., 2022).

Pezeshk et al. (2019) investigated the effectiveness of the warm water fish electric yellow Cichlid (*Labidochromis caeruleus*) fed with extracts obtained from the brown algae *Sargassum boveanum*, the red algae *Gracilaria persica* and the green algae *Entromorpha intestinalis* against low temperature stress. In the study, the fish were lowered from 26 degrees to 10 degrees and kept in this way for 30 minutes. At the end of the 56-day study, it was reported that the survival rates in the macroalgae supplemented groups were higher than the control (25%), and the highest rate was observed in *Entromorpha intestinalis* (75%). In addition, it has been reported that the growth performance of macroalgae added groups is increased and the time to reach the market length is shortened. The resistance of macroalgae to heat stress has been attributed to the fact that its bioactive compounds have increased the antioxidant capacity by reducing the formation of free radicals in the tissues.

Schleder et al. (2017) suggested that feeding on diets containing 0.5% and 2% *Sargassum filipendula* can provide an advantage for shrimp farming in regions where the temperature is unstable, by increasing the resistance of shrimp to thermal changes, resulting in a higher survival rate.

In another study, it was reported that up to 10% supplementation of AquaArom, extracted from brown seaweed Laminaria sp., kelp, increased

resistance to heat stress as well as, increased food intake, improved growth and antioxidant defense in Atlantic salmon (*Salmo salar*). Therefore, it has been suggested that it can be used to alleviate the negative effects of stress factors such as temperature in fish (Kamunde et al., 2019).

Use of macroalgae to reduce the effects of crowding stress:

Stock density, which is one of the most important factors determining the production of a fish farm, defines the amount of fish stocked per unit area (El-Sayed, 2006). Stock density affects survival, growth, behavior, health, water quality, nutrition and production. Low levels of stocking density increase efficiency, while at high levels, increased stocking density can reduce productivity (Herrera et al., 2015). As high stocking density can increase competition for space and access to food among fish, it can adversely affect the water quality, growth and survival rates of the aquatic organisms. Adverse physiological consequences can lead to inhibition of antioxidant defense and immunity, disease outbreaks, and eventual death (Silva-Brito et al. 2020; Negm et al., 2021). The choice of stock densities of fish is partly dependent on economic factors and market demands. In addition, environmental conditions such as feed quality, culture system, species and sex, and breeding techniques can affect optimal stocking density (El-Sayed, 2006). Optimum stock density is the level at which maximum yield is achieved. Therefore, stock density is an important indicator that determines the economic viability of the production system (Herrera et al., 2015).

Promising results have been achieved in reducing the stress caused by high stocking density in aquaculture. Safavi et al. (2019) reported that the inclusion of sulfated polysaccharides (1.5 g/kg) extracted from *Gracilariopsis persica* in the diet reduced the effects of crowded stress by providing a decrease in cortisol levels in rainbow trout (*Oncorhynchus mykiss*) exposed to stress compared to the control group. In addition, they have observed that fish fed with diets with macroalgae and exposed to crowded stress have increased growth performance, improved immune response antioxidant defense compared to the control group.

Siva-Brito et al. (2020) showed that dietary supplementation with 2.5% Gracilaria by-products can be used as a dietary tool to reduce oxidative stress while improving immune response in sea bream exposed to a crowding stressor. Therefore, It has been suggested that the use of macroalgae by-products in aquaculture may be an advantageous method with the potential to apply against crowded stress.

Negm et al. (2021) claimed that *Sargassum aquifolium* dietary supplements significantly mitigate the negative effects of high stock density

on cytokines in Nile Tilapia (*Oreochromis niloticus*), especially at 50 g / kg level. When applied between 50 and 100 g / kg, they suggested that it alleviated excessive crowded stress, oxidative stress and immunosuppression caused by high stocking density. In the study, it was determined that Tilapia diets enriched with diet from 100 to 200 g / kg Sargassum reduce the level of ammonia releasing in environment water.

Conclusion and future perspectives

Aquaculture appications are carried out with high stock density as a necessity of economic and environmental reasons. The emergence of physical, chemical, biological and procedural stressors are inevitable in such aquaculture systems and they always negatively affect sustainable production. Therefore, stress management in farmed fish is extremely important to optimize productivity. Stress management is performed in two fundamental categories, either over the species or over the culture environment. The main goal in these studies is to eliminate the destructive effect of stress, or at least to alleviate it. In the process of adding various functional feed additives to the diet, successful results have been achieved such as providing resistance to stress, increasing growth performance, improving immune system and antioxidant defense. However, these researches have been carried out mostly in small-scale areas, and there is a need for studies on the results in the aquaculture environment. In the aquaculture environment, studies should be performed to minimize the stress factors that each fish and other aquatic species may encounter in all life stages.

The fact that macroalgae have many bioactive contents such as polysaccharides and polyphenols, having many still unexplored species and being sustainable offer us important opportunities. Many stress factors provoke results that increase the effects of each other. The heat stress that arises as a result of global warming also leads to a decrease in the amount of dissolved oxygen and an increase in ammonia concentration in the environment. Similarly, high stock density causes a decrease in the amount of dissolved oxygen and an increase in the amount of ammonia. Therefore, it is required studies in which all stress factors will be evaluated together. Since the response and tolerance of aquatic organisms to stress may differ, diverse research plans should be designed for each species.

Furthermore, stress mitigation researches have focused on species that usually reared in Europe and the USA. However, most of the cultivation is performed in the Asian continent. Therefore, more studies should be focused on relieving stress factors encountered in the cultivation of species native to this continent.

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CHAPTER 8

DROUGHT ANALYSIS IN THE IZMIR PROVINCE BASED ON THE STANDARD PRECIPITATION INDEX (SPI)

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1. Introduction

Drought is a meteorological natural hazard that has great negative effects on the life of living things, limits various activities of people, and causes important ecological problems (Şahin and Sipahioğlu, 2003). Therefore, in order to objectively assess drought occurrence and better understand recent climatic effects, it would be useful to examine long-term precipitation series in regions without homogeneous climatic conditions. Because drought has negative consequences in some parts of the world with its increasing effect. There is no single definition of drought in the literature. Since the types and effects of drought differ from region to region, its definition may vary according to the region and sectors. The definition of drought is different for each discipline. In the simplest and general sense, drought is defined as "the situation where the water supply cannot meet the demand" in the relationship between supply and demand (Kadıoğlu, 2012). Drought has become a global problem today. Compared to other disasters, drought can adversely affect many natural and human resources with the uncertainty and increasing effect of the start and end times. Drought is directly related to precipitation and evaporation. Precipitation is the main factor affecting water availability for many systems, as it varies greatly regionally and temporally. Therefore, many drought indices are mainly based on precipitation conditions (Pamuk et al. 2004). However, it is not correct to say that only a decrease in the amount of precipitation can directly cause drought (Şahin and Sipahioğlu, 2003). "In order to decide on the drought, the temperature, precipitation amount and precipitation regime and the evaporation conditions depending on the ground feature should be taken into consideration together" (Şahin and Sipahioğlu, 2003). The Standardized Precipitation Index (SPI), which converts the precipitation parameter into a single numerical value in order to analyze the drought of areas with different climates in drought studies, was developed by McKee et al. (1993) to describe and monitor drought. The Standard Precipitation Index (SPI) method is obtained by dividing the difference of precipitation (Xi) from the mean (Xi mean) by the standard deviation (σ) in a selected time period. During the literature review, it was seen that this method was used in some studies conducted in our country. Caldag et al. (2004) (for the Thrace region) used the SPI drought method in their study. According to the SPI values they obtained, they concluded that the Thrace region was under the influence of severe drought between 2000-2001, except for Istanbul. Topçuoğlu et al. (2008) made a drought analysis for the Aegean region using the SPI method within 1,3 and 12 month periods. As a result of the analysis, they concluded that drought was observed in the region in 1977, 1989, 1990 and 1992. Ilgar (2010) made a drought analysis for Çanakkale by using the long annual precipitation data for the years 1929-2007, using

the SPI method within 3 and 12 month periods. Kiymaz et al. (2011) used the SPI method to analyze the drought occurrences for Seyfe lake for 1, 3, 6, 12, and 24 month periods as the first period (1975-1991) and the second period (1992-2008). As a result of their analysis, they concluded that severe and very severe droughts show minimum values for short and long periods in both periods. Silver et al. (2016) conducted a drought analysis for the province of Şanlıurfa for 1, 3, 6 and 12-month periods using the SPI method, using 78 years of precipitation data between 1937-2014. As a result of the analysis, they stated that the number of months with 29 years of extreme dryness between 1986 and 2014 was higher than the number of months with 49 years of dryness between the years 1937-1985. Karaer and Gültaş (2018) conducted a drought analysis for Bilecik province using long-term precipitation data between 1980-2014 (within 1, 3, 6, 12, 24 month periods) using the SPI method. As a result of their analysis, they determined that the drought was felt most in the 6 and 12-month periods, and the very severe drought occurred in the 12-month period in 1985. In the seasonal evaluation, they found that drought is generally seen in summer months, but also in winter months in some periods. In this study, 3, 6, and 12-month drought indices were calculated using the SPI method by using the monthly total precipitation values of Izmir station numbered 17220. The temporal changes of the drought index values were examined with the drought analysis made using the data between 1980-2019.

2. MATERIAL AND METHOD

2.1. Research area and data

The study was carried out in Izmir province, located in the western Anatolia, situated along the Aegean coast region in Turkey. It covers Izmir station numbered 17220 (Figure 1).

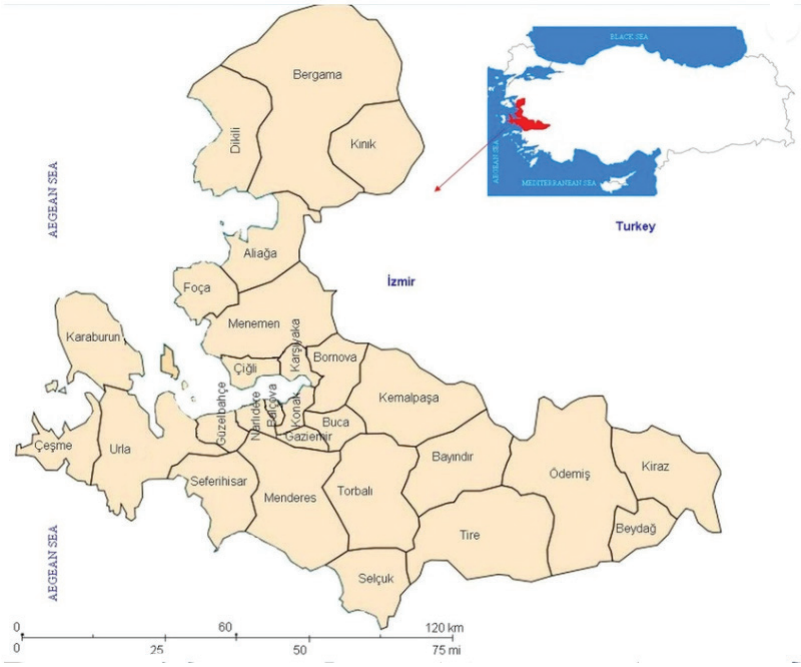

Figure 1. Location map of Izmir

Table 1: Izmir meteorological station informations

İstation name	istation No	altitude (m)	Observation Year	Latitude	Longitude
Izmir	17220	2	1980-2019	38.4333 N	27.1428 E

İzmir is a province located in the coastal areas of the Aegean region. Therefore, it is under the influence of the Mediterranean climate in terms of climate characteristics. For this reason, the summer months are hot and dry, while the winter months are warm and rainy. The hottest months in Izmir city are July and August. Precipitation in winter is mostly rain. Snowfall is extremely rare. Annual precipitation varies between 700-1000 mm depending on the regions (Table 2).

2.2 Method

2.2.1 Standard Precipitation Index

In the study, the Standardized Precipitation Index (SPI) method, which converts the precipitation parameter into a single numerical value, was used to define the drought of regions with different climates. This method was first described by **Mckee et al. (1993)**. This method is obtained

by dividing the difference of precipitation (Xi) from the mean (Xi mean) by the standard deviation (σ) in a selected time period with the following equation.

$$\textbf{Formul: } SPI = \frac{Xi - Xiort}{\sigma}$$

Table 2: SPI Drought Severity Classes (McKee et al.,1994).

SPI values	Drought Category
more than 2.0	Exceptionally Humid
1.60 to 1.99	Extremely Moist
1.30 to 1.59	Too Moist
0.80 to 1.29	Moderately Humid
0.51 to 0.79	Slightly moist
0.50 to -0.50	Around normal
-0.51 to -0.79	Mild Arid
-0.80 to -1.29	Medium Arid
-1.30 to -1.59	Severe Drought
-1.60 to -1.99	Very Severe Drought
-2.0 and lower	Extraordinary Arid

According to the drought severity classes in Table 2, if the SPI value drops below 0, it indicates that the area has entered the dry period. When it drops below -2.0, there is an extraordinary drought period in the field. The fact that this value is positive indicates that the drought period has started in the area around normal. According to the table, if the SPI value is more than 2.0, the site will experience extraordinary humidity. The results obtained in this study were evaluated according to the SPI drought severity classes (McKee et al., 1994) (Table 2).

3. FINDINGS

3.1. Drought Analysis

For drought analysis, monthly precipitation data measured between 1980 and 2020 at Izmir station 17220 located in the coastal areas of the Aegean Region of Turkey were used. SPI values were calculated according to the gamma distribution of the precipitation data using the DrinC program.

Table 3: 1-month SPI index values and drought classification

Years	SPI (Janury) Values 1 month	Drought Classification	SPI (February) Values 1 month	Drought Classification	SPI (March) Values 1 month	Drought Classification	SPI (April) Values 1 month	Drought Classification
1980	1.69	Extremely Moist	-0.49	Around normal	0.29	Around normal	-1.23	Medium Arid
1981	-0.56	Mild Arid	-0.47	Around normal	0.72	Slightly moist	1.37	Too Moist
1982	-0.83	Medium Arid	0.35	Around normal	-2.08	Extraordinary Arid	0.01	Around normal
1983	1.12	Moderately Humid	0.26	Around normal	0.63	Slightly moist	0.84	Moderately Humid
1984	0.38	Around normal	-0.90	Medium Arid	0.62	Slightly moist	-1.88	Very Severe Drought
1985	1.38	Too Moist	0.84	Moderately Humid	-0.96	Medium Arid	0.13	Around normal
1986	0.86	Moderately Humid	-0.42	Around normal	0.79	Slightly moist	0.19	Around normal
1987	-0.69	Mild Arid	0.31	Around normal	1.42	Too Moist	-0.70	Mild Arid
1988	-1.89	Very Severe Drought	-1.84	Very Severe Drought	0.98	Moderately Humid	-2.73	Extraordinary Arid
1989	-1.16	Medium Arid	0.07	Around normal	-1.34	Severe Drought	0.69	Slightly moist
1990	-1.06	Medium Arid	-0.38	Around normal	-1.21	Medium Arid	0.22	Around normal
1991	-1.96	Very Severe Drought	-1.47	Severe Drought	0.16	Around normal	-0.08	Around normal
1992	-0.67	Mild Arid	0.79	Slightly moist	0.42	Around normal	0.26	Around normal
1993	-0.46	Around normal	0.20	Around normal	0.19	Around normal	0.28	Around normal
1994	0.89	Moderately Humid	-1.09	Medium Arid	1.66	Extremely Moist	-0.03	Around normal
1995	-1.18	Medium Arid	1.35	Too Moist	-0.77	Mild Arid	1.23	Moderately Humid
1996	0.13	Aruond normal	-1.36	Severe Drought	0.92	Moderately Humid	1.28	Moderately Humid
1997	0.61	Slightly moist	-0.16	Around normal	1.04	Moderately Humid	-0.19	Around normal
1998	-0.16	Around nomal	1.65	Extremely Moist	0.18	Around normal	-1.17	Medium Arid
1999	-0.12	Around nomal	0.15	Around normal	0.18	Around normal	0.25	Around normal
2000	-0.19	Around nomal	0.37	Around normal	-1.64	Very Severe Drought	0.71	Slightly moist
2001	-1.08	Around nomal	-0.91	Medium Arid	0.41	Around normal	0.32	Around normal
2002	-0.05	Around nomal	1.28	Moderately Humid	-1.19	Medium Arid	1.48	Too Moist
2003	1.03	Moderately Humid	-1.10	Medium Arid	-1.37	Severe Drought	-0.34	Around normal
2004	0.18	Around normal	1.92	Extremely Moist	0.41	Around normal	-0.95	Medium Arid
2005	-0.36	Around normal	0.18	Around normal	1.60	Extremely Moist	-0.38	Around normal
2006	-1.12	Medium Arid	-1.28	Medium Arid	-1.02	Medium Arid	-0.84	Medium Arid

2007	-1.19	Medium Arid	-1.98	Very Severe Drought	-0.18	Around normal	0.62	Slightly moist
2008	0.86	Moderately Humid	0.97	Moderately Humid	1.55	Too Moist	1.09	Moderately Humid
2009	0.35	Around normal	2.01	Exceptionally Humid	-1.63	Very Severe Drought	-0.78	Mild Arid
2010	-0.07	Around normal	0.36	Around normal	-1.49	Severe Drought	0.69	Slightly moist
2011	0.21	Around normal	0.60	Slightly moist	-0.85	Medium Arid	1.49	Too Moist
2012	1.19	Around normal	1.17	Moderately Humid	-0.25	Around normal	-0.34	Around normal
2013	0.42	Around normal	-1.62	Very Severe Drought	0.66	Slightly moist	1.92	Extremely Moist
2014	0.63	Slightly moist	0.27	Around normal	0.69	Slightly moist	-0.30	Around normal
2015	1.06	Around normal	0.19	Around normal	0.89	Moderately Humid	-0.42	Around normal
2016	1.40	Too Moist	-0.65	Mild Arid	0.90	Moderately Humid	-0.79	Mild Arid
2017	-0.11	Around normal	0.63	Slightly moist	-0.37	Around normal	-2.22	Extraordinary Arid
2018	2.06	Exceptionally Humid	0.37	Around normal	-0.64	Mild Arid	0.30	Around normal
2019	-0.98	Medium Arid	-0.17	Around normal	-0.21	Around normal	0.33	Around normal
2020	-0.98	Medium Arid	-0.17	Around normal	-0.21	Around normal	0.33	Around normal

Years	**SPI (May) Values 1 month**	**Drought Classification**	**SPI (June) Values 1 month**	**Drought Classification**	**SPI (Julay) Values 1 month**	**Drought Classification**	**SPI (August) Values 1 month**	**Drought Classification**
1980	0.10	Around normal	-0.76	Mild Arid	0.67	Slightly moist	0.13	Around normal
1981	0.97	Moderately Humid	-0.76	Mild Arid	0.81	Moderately Humid	0.13	Around normal
1982	-1.43	Severe Drought	0.59	Slightly moist	1.60	Extremely Moist	0.42	Around normal
1983	-2.50	Extraordinary Arid	-0.76	Mild Arid	1.98	Extremely Moist	0.70	Slightly moist
1984	0.73	Slightly moist	-0.24	Around normal	0.67	Slightly moist	0.13	Around normal
1985	-0.79	Mild Arid	0.92	Moderately Humid	0.67	Slightly moist	0.13	Around normal
1986	-1.00	Medium Arid	-0.24	Around normal	0.91	Moderately Humid	0.13	Around normal
1987	-0.71	Mild Arid	-0.76	Mild Arid	0.67	Slightly moist	0.13	Around normal
1988	0.37	Around normal	1.41	Too Moist	0.67	Slightly moist	0.13	Around normal
1989	-1.17	Medium Arid	-0.09	Around normal	0.67	Slightly moist	0.72	Slightly moist
1990	1.87	Extremely Moist	-0.76	Mild Arid	2.54	Exceptionally Humid	0.29	Around normal
1991	-0.59	Mild Arid	0.05	Around normal	0.67	Slightly moist	0.68	Slightly moist

1992	0.39	Around normal	-0.62	Mild Arid	0.67	Slightly moist	0.13	Around normal
1993	0.45	Around normal	-0.27	Around normal	0.67	Slightly moist	0.42	Around normal
1994	0.56	Slightly moist	-0.76	Mild Arid	0.67	Slightly moist	1.27	Moderately Humid
1995	-0.10	Around normal	-0.62	Mild Arid	0.67	Slightly moist	0.39	Around normal
1996	-0.37	Around normal	-0.16	Around normal	1.04	Moderately Humid	0.13	Around normal
1997	2.19	Exceptionally Humid	-0.76	Mild Arid	0.67	Slightly moist	0.13	Around normal
1998	-0.37	Around normal	-0.39	Around normal	0.67	Slightly moist	0.13	Around normal
1999	-1.00	Medium Arid	-0.76	Mild Arid	0.67	Slightly moist	0.29	Around normal
2000	-0.09	Around normal	0.77	Slightly moist	0.99	Moderately Humid	2.30	Exceptionally Humid
2001	-0.55	Mild Arid	-0.76	Mild Arid	0.67	Slightly moist	0.13	Around normal
2002	-0.54	Mild Arid	-0.62	Mild Arid	0.88	Moderately Humid	0.13	Around normal
2003	-0.47	Around normal	0.03	Around normal	0.67	Slightly moist	0.13	Around normal
2004	0.55	Slightly moist	0.91	Moderately Humid	0.67	Slightly moist	0.35	Around normal
2005	-2.50	Extraordinary Arid	0.46	Around normal	0.67	Slightly moist	0.13	Around normal
2006	0.78	Slightly moist	-0.52	Mild Arid	0.67	Slightly moist	0.13	Around normal
2007	-1.03	Medium Arid	-0.48	Around normal	0.67	Slightly moist	0.42	Around normal
2008	0.78	Slightly moist	0.41	Around normal	0.67	Slightly moist	0.13	Around normal
2009	0.27	Around normal	2.15	Exceptionally Humid	0.67	Slightly moist	0.13	Around normal
2010	0.35	Around normal	-0.42	Around normal	0.67	Slightly moist	0.13	Around normal
2011	1.64	Extremely Moist	0.88	Moderately Humid	0.67	Slightly moist	0.13	Around normal
2012	0.77	Slightly moist	1.11	Moderately Humid	0.67	Slightly moist	1.80	Extremely Moist
2013	-0.24	Around normal	1.64	Extremely Moist	0.85	Moderately Humid	0.91	Moderately Humid
2014	0.32	Around normal	1.71	Extremely Moist	0.67	Slightly moist	2.32	Exceptionally Humid
2015	0.59	Slightly moist	-0.07	Around normal	0.67	Slightly moist	0.42	Around normal
2016	0.82	Moderately Humid	-0.01	Around normal	0.67	Slightly moist	0.29	Around normal
2017	0.07	Around normal	0.59	Slightly moist	0.67	Slightly moist	0.52	Slightly moist
2018	-0.35	Around normal	0.99	Moderately Humid	0.84	Moderately Humid	0.13	Around normal
2019	1.52	Slightly moist	1.41	Too Moist	0.67	Slightly moist	0.13	Around normal
2020	1.52	Slightly moist	1.41	Too Moist	0.67	Slightly moist	0.13	Around normal

Years	SPI (Sep) Values 1 month	Drought Classification	SPI (Oct) Values 1 month	Drought Classification	SPI (Nov) Values 1 month	Drought Classification	SPI (Dec) Values 1 month	Drought Classification
1980	-0.19	Around normal	-0.76	Mild Arid	0.86	Moderately Humid	0.84	Moderately Humid
1981	-0.19	Around normal	-0.25	Around normal	1.11	Moderately Humid	1.93	Extremely Moist
1982	-0.19	Around normal	0.89	Moderately Humid	-0.27	Around normal	0.17	Around normal
1983	-0.14	Around normal	-1.23	Medium Arid	0.96	Moderately Humid	0.48	Around normal
1984	-0.19	Around normal	-1.65	Very Severe Drought	-0.02	Around normal	-0.81	Medium Arid
1985	-0.08	Around normal	0.00	Around normal	0.75	Slightly moist	-1.64	Very Severe Drought
1986	-0.19	Around normal	-0.21	Around normal	-1.59	Severe Drought	0.09	Around normal
1987	-0.19	Around normal	-1.37	Severe Drought	1.06	Moderately Humid	0.38	Around normal
1988	0.86	Moderately Humid	-0.98	Medium Arid	0.43	Around normal	0.39	Around normal
1989	0.31	Around normal	0.33	Around normal	-0.11	Around normal	0.36	Around normal
1990	-0.19	Around normal	-0.29	Around normal	-1.56	Severe Drought	1.45	Too Moist
1991	-0.19	Around normal	-0.49	Around normal	-1.29	Medium Arid	0.11	Around normal
1992	0.07	Around normal	-0.68	Mild Arid	0.32	Around normal	-0.27	Around normal
1993	-0.19	Around normal	-0.74	Mild Arid	0.28	Around normal	0.66	Slightly moist
1994	0.68	Slightly moist	0.76	Slightly moist	-0.11	Around normal	0.44	Around normal
1995	2.00	Exceptionally Humid	-1.10	Medium Arid	1.16	Moderately Humid	-0.42	Around normal
1996	-0.19	Around normal	0.24	Around normal	-0.40	Around normal	0.81	Moderately Humid
1997	1.06	Moderately Humid	0.41	Around normal	-0.12	Around normal	0.91	Moderately Humid
1998	-0.11	Around normal	0.97	Moderately Humid	2.10	Exceptionally Humid	0.46	Around normal
1999	-0.19	Around normal	-0.02	Around normal	-0.88	Medium Arid	0.15	Around normal
2000	0.73	Slightly moist	1.07	Moderately Humid	0.27	Around normal	-1.28	Medium Arid
2001	1.46	Too Moist	-1.65	Very Severe Drought	2.21	Exceptionally Humid	1.39	Too Moist
2002	-0.19	Around normal	1.01	Moderately Humid	0.54	Slightly moist	0.38	Around normal
2003	-0.19	Around normal	0.72	Slightly moist	-1.78	Very Severe Drought	0.02	Around normal
2004	0.25	Around normal	-1.34	Severe Drought	0.15	Around normal	-0.49	Around normal
2005	2.51	Exceptionally Humid	-0.19	Around normal	0.93	Moderately Humid	-0.66	Mild Arid
2006	-0.19	Around normal	1.34	Too Moist	-0.53	Mild Arid	-1.89	Very Severe Drought

2007	1.30	Too Moist	1.27	Moderately Humid	0.33	Around normal	0.05	Around normal
2008	1.25	Moderately Humid	-0.60	Mild Arid	0.03	Around normal	-0.17	Around normal
2009	0.45	Around normal	-0.09	Around normal	0.98	Moderately Humid	0.41	Around normal
2010	0.33	Around normal	2.39	Exceptionally Humid	-1.30	Severe Drought	0.45	Around normal
2011	-0.19	Around normal	1.05	Moderately Humid	-1.96	Very Severe Drought	0.29	Around normal
2012	0.19	Around normal	-0.21	Around normal	-0.67	Mild Arid	1.00	Moderately Humid
2013	0.40	Around normal	1.10	Moderately Humid	0.58	Slightly moist	-1.89	Very Severe Drought
2014	0.27	Around normal	1.07	Moderately Humid	-1.34	Severe Drought	1.24	Moderately Humid
2015	0.33	Around normal	0.97	Moderately Humid	0.16	Around normal	-1.96	Very Severe Drought
2016	-0.19	Around normal	-1.53	Severe Drought	0.51	Slightly moist	-1.65	Very Severe Drought
2017	0.16	Around normal	0.64	Slightly moist	-0.56	Mild Arid	0.04	Around normal
2018	0.94	Moderately Humid	0.29	Around normal	-0.26	Around normal	0.09	Around normal
2019	-0.19	Around normal	-0.18	Around normal	-0.45	Around normal	-0.22	Around normal
2020	-0.19	Around normal	-0.18	Around normal	-0.45	Around normal	-0.22	Around normal

When Table 3 is examined, according to the spring season 1-month (March, April, May) SPI index values, mostly around normal drought class (0.50 , -50) is seen in the region, and in some periods, Extraodinary dry, Severe drought, moderately dry and mildly dry periods are seen for some periods. In some years, Exeptionally humid, too moist, extremely moist, moderately humid and slightly humid periods were also observed. According to the 1-month SPI index values of the summer season, mostly around normal drought (0.50, -50) and slightly moist (0.51, 0.79) was experienced in the region. Remarkably, Exeptionally humid, extremely humid, moderately humid and too humid periods were experienced in the summer. According to the 1-month SPI index values of the summer season, a drought period was generally Mild dry. According to the 1-month SPI index values of the autumn season SPI index values, mostly around normal drought class (0.50, -0.50) it is seen that the drought is classified in class of very severe drought, and while there are short- term, mild dry and medium dry periods in some periods, humid periods are experienced in the autumn season. According to the 1-month SPI index values of the winter season, around normal drought is observed, the humid periods and dry periods are also observed in winter.

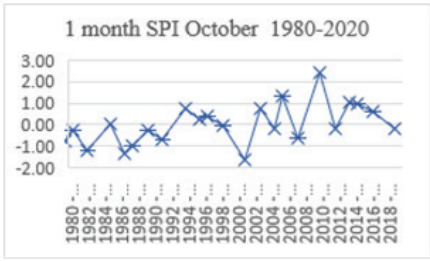

Figure 2: 1 month SPI October graphics

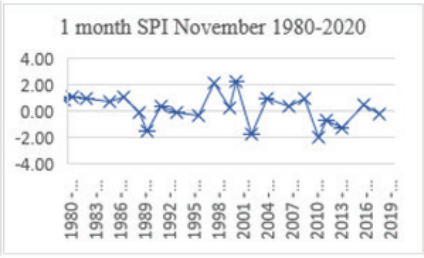

Figure 3: 1 month SPI November graphics

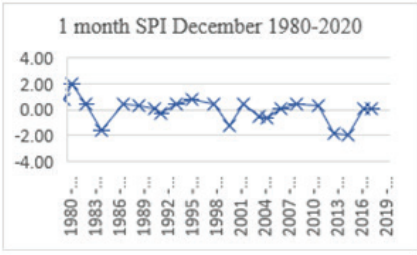

Figure 4: 1 month SPI December graphics

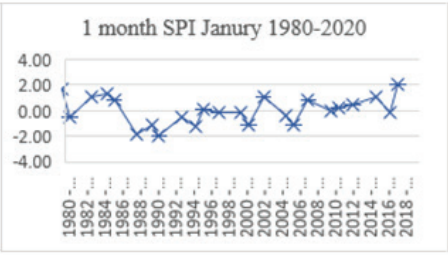

Figure 5: 1 month SPI Janury graphics

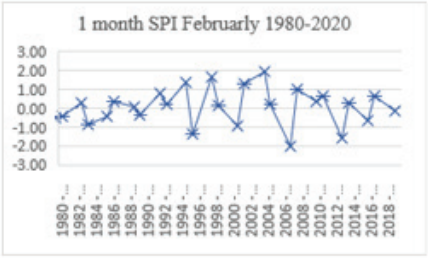

Figure 6: 1 month SPI February graphics

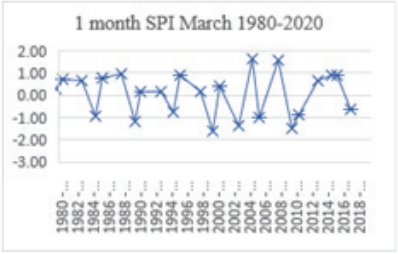

Figure 7: 1 month SPI March graphics

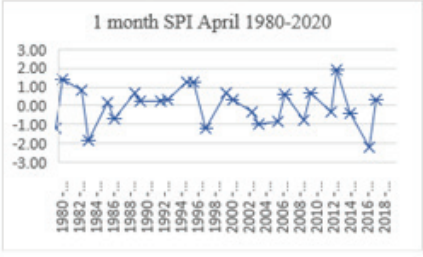

Figure 8: 1 month SPI April graphics

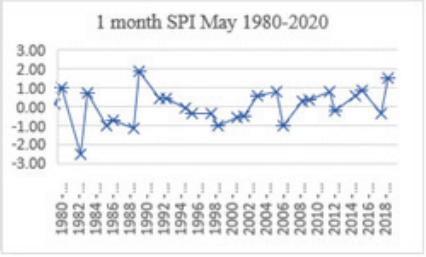

Figure 9: 1 month SPI May graphics

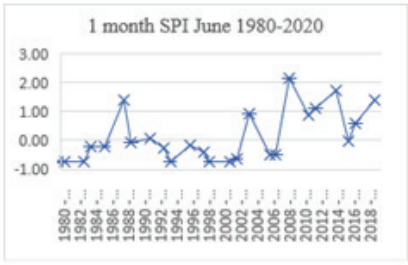

Figure 10: 1 month SPI June graphics

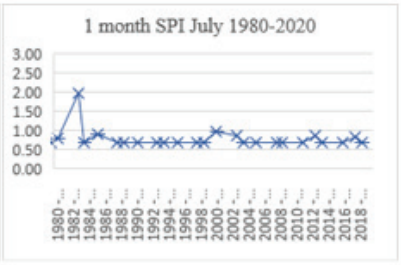

Figure 11: 1 month SPI July graphics

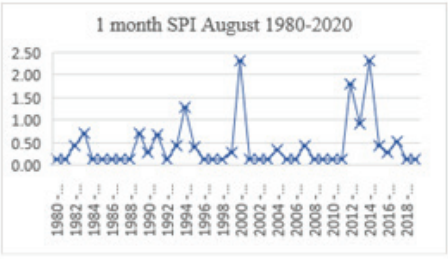

Figure 12: 1 month SPI August graphics

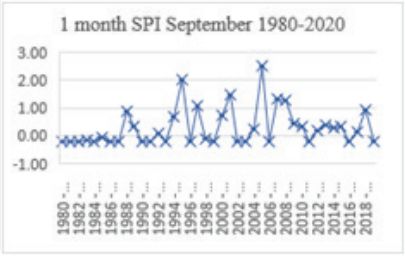

Figure 13: 1 month SPI September graphics

Table 4: 3-month SPI index values and drought classification

Years	SPI (M,A,M) Values 3 month	Drought Classification	SPI (J,J,A) Values 3 month	Drought Classification	SPI (S,O,N) Values 3 month	Drought Classification	SPI (D,J,F) Values 3 month	Drought Classification
1980	0.86	Moderately Humid	1.13	Moderately Humid	-1.46	Severe Drought	-0.84	Medium Arid
1981	2.35	Exceptionally Humid	-0.48	Around normal	1.37	Too Moist	-0.55	Mild Arid
1982	0.14	Around normal	-1.09	Medium Arid	-0.72	Mild Arid	0.15	Around normal
1983	0.51	Slightly Moist	0.98	Moderately Humid	-0.25	Around normal	0.44	Around normal
1984	-1.60	Very Severe Drought	-0.03	Around normal	-0.90	Medium Arid	-0.84	Medium Arid
1985	-0.94	Medium Arid	1.09	Moderately Humid	-0.23	Around normal	-0.55	Mild Arid
1986	-1.30	Severe Drought	0.57	Slightly Moist	-0.81	Medium Arid	-0.42	Around normal
1987	0.50	Around normal	0.24	Around normal	-1.72	Very Severe Drought	-0.84	Medium Arid
1988	0.04	Around normal	-1.30	Severe Drought	-0.28	Around normal	0.71	Slightly Moist
1989	0.06	Around normal	-1.37	Severe Drought	-0.26	Around normal	0.14	Around normal
1990	0.54	Slightly Moist	-1.62	Very Severe Drought	1.32	Too Moist	0.68	Slightly Moist
1991	-1.25	Medium Arid	-1.98	Very Severe Drought	-0.87	Medium Arid	-0.40	Around normal
1992	-0.65	Mild Arid	0.04	Around normal	-0.05	Around normal	-0.28	Around normal

1993	0.27	Around normal	-0.34	Around normal	0.04	Around normal	-0.62	Mild Arid
1994	0.40	Around normal	0.85	Moderately Humid	-0.16	Around normal	0.72	Slightly Moist
1995	-0.08	Around normal	-0.08	Around normal	0.61	Slightly Moist	1.89	Extremely Moist
1996	0.36	Around normal	-0.19	Around normal	0.60	Slightly Moist	-0.84	Medium Arid
1997	0.70	Slightly Moist	0.57	Slightly Moist	1.46	Too Moist	0.97	Moderately Humid
1998	2.11	Exceptionally Humid	0.84	Moderately Humid	-1.85	Very Severe Drought	-0.62	Mild Arid
1999	-0.78	Mild Arid	-0.17	Around normal	-0.79	Mild Arid	-0.73	Mild Arid
2000	-0.40	Around normal	-0.58	Mild Arid	0.46	Around normal	1.22	Moderately Humid
2001	2.40	Exceptionally Humid	-1.13	Medium Arid	-0.56	Mild Arid	1.37	Too Moist
2002	0.90	Moderately Humid	0.28	Around normal	0.73	Slightly Moist	-0.84	Medium Arid
2003	-0.86	Medium Arid	-0.08	Around normal	-1.07	Medium Arid	-0.47	Around normal
2004	-1.12	Medium Arid	1.27	Moderately Humid	-0.19	Around normal	-0.01	Around normal
2005	-0.26	Around normal	0.43	Around normal	-1.29	Medium Arid	2.40	Exceptionally Humid
2006	-1.02	Medium Arid	-2.13	Extraodinary Drought	-0.47	Around normal	-0.84	Medium Arid
2007	0.69	Slightly Moist	-1.97	Very Severe Drought	-0.36	Around normal	1.19	Moderately Humid
2008	-0.76	Mild Arid	1.48	Too Moist	1.15	Moderately Humid	1.12	Moderately Humid
2009	0.69	Slightly Moist	1.05	Moderately Humid	0.90	Moderately Humid	0.23	Around normal
2010	1.41	Too Moist	-0.48	Around normal	0.32	Around normal	0.08	Around normal
2011	-0.45	Around normal	0.01	Around normal	2.27	Exceptionally Humid	-0.84	Medium Arid
2012	0.29	Around normal	1.24	Moderately Humid	0.45	Around normal	0.62	Slightly Moist
2013	-0.43	Around normal	-0.13	Around normal	2.06	Exceptionally Humid	0.34	Around normal
2014	0.99	Moderately Humid	0.63	Slightly Moist	0.68	Slightly Moist	0.97	Moderately Humid
2015	-1.05	Medium Arid	1.00	Moderately Humid	-0.34	Around normal	0.10	Around normal
2016	-1.66	Very Severe Drought	1.02	Moderately Humid	-0.31	Around normal	-0.73	Mild Arid
2017	-0.32	Around normal	-0.06	Around normal	-1.34	Severe Drought	-0.10	Around normal
2018	-0.33	Around normal	1.57	Too Moist	0.12	Around normal	0.81	Moderately Humid
2019	-1.01	Medium Arid	-1.03	Medium Arid	1.66	Extremely Moist	-0.84	Medium Arid
2020	-1.01	Medium Arid	-1.03	Medium Arid	1.66	Extremely Moist	-0.84	Medium Arid

When Table 4 is examined, according to the spring season 3-month (March, April, May) SPI index values, some periods classified around normal drought class (0.50 , -50) is seen in the region, and in some periods, very severe drought, severe drought, Medium dry periods are seen for some periods. In some years, Exceptionally humid, moderately humid and slightly humid periods were also observed. According to the 3-month SPI index values of the summer season, some periods classified around normal drought (0.50, -50) was experienced in the region. Remarkably, only Exceptionally humid period was experienced in the summer in 2011 and 2013. According to the 3-month SPI index values of the summer season, a drought period was generally classified Extraordinary drought, very severe drought, severe drought, Mild Arid and Medium Arid. In the summer season the humid periods are classified as too moist, moderately humid and slightly moist. According to the 3-month SPI index values of the autumn season, it is seen that the drought is classified as very severe drought, severe drought, mild dry and medium dry. Humid periods are experienced as Extremely moist, Moderately humid and too moist in the autumn period. According to the 3-month SPI index values of the winter season, generally around normal drought is observed. Mild Arid and Medium dry periods are observed in winter. Humid period is seen in winter and classified as Exceptionally humid, Too humid, Extremely humid, Moderately humid and slightly humid.

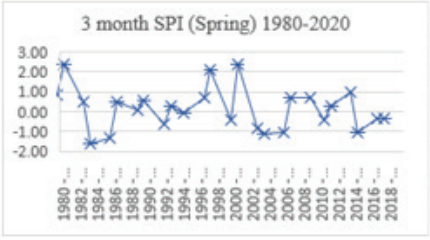

Figure 14: 3 month (Spring) SPI graphics

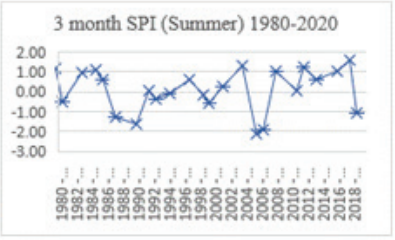

Figure 15: 3 month (Summer) SPI graphics

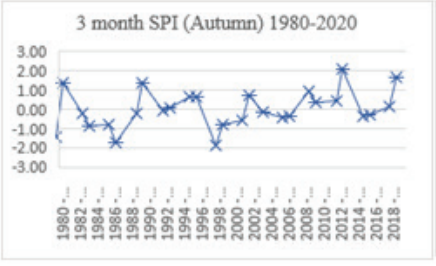

Figure 16: 3 month (Autumn) SPI graphics

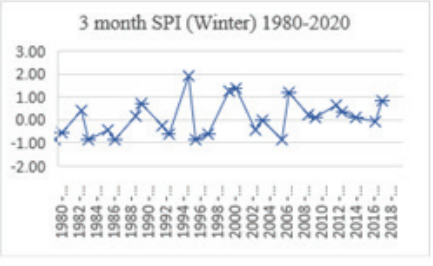

Figure 17: 3 month (Winter) SPI graphics

Table 5: SPI index values for 6-9- 12 months and drought classification.

Years	SPI (M-A-M-J-J-A) Values 6 Months	Drought Classification	SPI (S-O-N-D-J-F) Values 6 Months	Drought Classification	SPI (J-F-M-A-M-J-J-A-S-O-N-D) Values 9 Months	Drought Classification	SPI (J-F-M-A-M-J-J-A-S-O-N-D) Values 12 Months	Drought Classification
1980	1.39	Too Moist	-1.62	Very Severe Drought	1.09	Moderately Humid	0.94	Moderately Humid
1981	1.10	Moderately Humid	0.82	Moderately Humid	1.36	Too Moist	1.22	Moderately Humid
1982	-0.86	Medium Arid	-0.72	Mild Arid	-1.05	Medium Arid	-1.07	Medium Arid
1983	1.06	Moderately Humid	-0.18	Around normal	0.94	Moderately Humid	0.89	Moderately Humid
1984	-0.97	Medium Arid	-1.14	Medium Arid	-1.19	Medium Arid	-1.27	Medium Arid
1985	0.49	Around normal	-0.54	Mild Arid	0.38	Around normal	0.26	Around normal
1986	-0.21	Around normal	-1.02	Medium Arid	-0.43	Around normal	-0.53	Mild Arid
1987	0.39	Around normal	-1.84	Very Severe Drought	0.04	Around normal	-0.07	Around normal
1988	-1.08	Medium Arid	0.00	Around normal	-1.15	Medium Arid	-1.03	Medium Arid
1989	-1.11	Medium Arid	-0.36	Around normal	-1.17	Medium Arid	-1.19	Medium Arid
1990	-0.91	Medium Arid	1.15	Moderately Humid	-0.47	Around normal	-0.40	Around normal
1991	-2.44	Extraordinary Drought	-1.01	Medium Arid	-2.59	Extraordinary Drought	-2.63	Extraordinary Drought
1992	-0.43	Around normal	-0.34	Around normal	-0.47	Around normal	-0.55	Mild Arid
1993	-0.23	Around normal	-0.31	Around normal	-0.25	Around normal	-0.36	Around normal
1994	0.88	Moderately Humid	1.10	Moderately Humid	0.78	Slightly Moist	0.79	Slightly Moist
1995	-0.22	Around normal	1.69	Extremely Moist	-0.09	Around normal	0.42	Around normal
1996	-0.05	Around normal	0.16	Around normal	0.07	Around normal	-0.05	Around normal
1997	0.80	Moderately Humid	1.43	Too Moist	1.11	Moderately Humid	1.16	Moderately Humid
1998	1.86	Extremely Moist	-1.94	Very Severe Drought	1.53	Too Moist	1.38	Too Moist
1999	-0.70	Mild Arid	-1.04	Medium Arid	-0.90	Medium Arid	-1.00	Medium Arid
2000	-0.83	Medium Arid	0.94	Moderately Humid	-0.70	Mild Arid	-0.44	Around normal
2001	0.79	Slightly Moist	0.50	Around normal	0.61	Slightly Moist	0.83	Moderately Humid
2002	0.66	Slightly Moist	0.27	Around normal	0.77	Slightly Moist	0.64	Slightly Moist
2003	-0.65	Mild Arid	-1.25	Medium Arid	-0.91	Medium Arid	-1.00	Medium Arid
2004	0.61	Slightly Moist	-0.37	Around normal	0.51	Slightly Moist	0.42	Around normal
2005	0.14	Around normal	1.53	Too Moist	-0.16	Around normal	0.64	Slightly Moist
2006	-2.37	Extraordinary Drought	-0.76	Mild Arid	-2.40	Extraordinary Drought	-2.46	Extraordinary Drought

2007	-0.93	Medium Arid	0.40	Around normal	-1.03	Medium Arid	-0.75	Mild Arid
2008	0.98	Moderately Humid	1.31	Too Moist	1.19	Moderately Humid	1.28	Moderately Humid
2009	1.23	Moderately Humid	0.61	Slightly Moist	1.35	Too Moist	1.26	Moderately Humid
2010	0.43	Around normal	0.08	Around normal	0.44	Around normal	0.36	Around normal
2011	-0.35	Around normal	1.58	Too Moist	0.39	Around normal	0.26	Around normal
2012	1.20	Moderately Humid	0.45	Around normal	1.22	Moderately Humid	1.20	Moderately Humid
2013	-0.47	Around normal	1.58	Too Moist	0.20	Around normal	0.16	Around normal
2014	1.01	Moderately Humid	0.88	Moderately Humid	1.09	Moderately Humid	1.15	Moderately Humid
2015	0.35	Around normal	-0.45	Around normal	0.22	Around normal	0.14	Around normal
2016	0.14	Around normal	-0.63	Mild Arid	0.02	Around normal	-0.10	Around normal
2017	-0.34	Around normal	-1.33	Severe Drought	-0.65	Mild Arid	-0.72	Mild Arid
2018	1.25	Moderately Humid	0.36	Around normal	1.20	Moderately Humid	1.21	Moderately Humid
2019	-1.58	Severe Drought	1.06	Moderately Humid	-0.89	Medium Arid	-0.99	Medium Arid
2020	-1.58	Severe Drought	1.06	Moderately Humid	-0.89	Medium Arid	-0.99	Medium Arid

When Table 5 is examined, as a result of the 6-month index values calculated according to the Izmir meteorological station (1980-2020) values, it is seen that normal drought, Too moist, Moderately humid, Medium Arid, Extraordinary drought, Extremely moist, Mild Arid, Severe drought and slightly moist are experienced (M-A-M-J-J-A). As a result of the 6-month index values of the winter months (S-O-N-D-J-F), it is seen that the drought periods are, very severe drought, severe drought, Medium arid and Mild arid are observed. In some term, too moist, moderately humid, Extremely moist and slightly moist periods are observed in some years. As a result of the 9-month index values calculated according to the data of Izmir meteorology station (1980-2020), drought around normal has been experienced in 14 years, Moderately humid has been experienced in 7 years, Too moist has been experienced in 3 years, Medium Arid has been experienced in 9 years, Extraordinary drought has been experienced in 2006 and 1991, slightly moist has been experienced in 4 years, Mild Arid has been experienced in 2000 and 2017. As a result of the 12-month index values calculated according to the data of Izmir meteorology station (1980-2020), Around normal drought are seen in 13 years, Moderately humid are seen in 10 years, Too moist is seen in 1998, Medium Arid are seen in 8 years, Extraordinary drought are seen in 2006 and 1991, Slightly moist are seen in 3 years and Mid Arid are seen in 4 years.

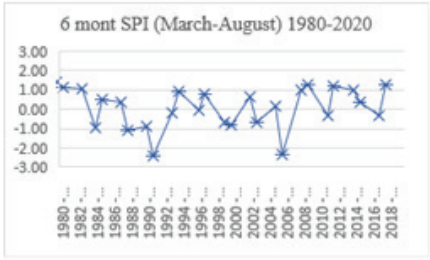

Figure 18: 6 month (March-August) SPI graphics

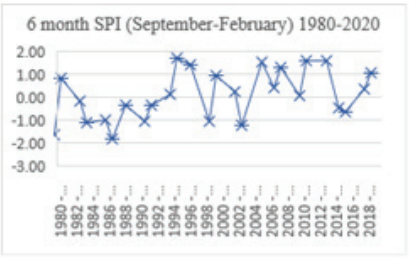

Figure 19: 6 month (September-February) SPI graphics

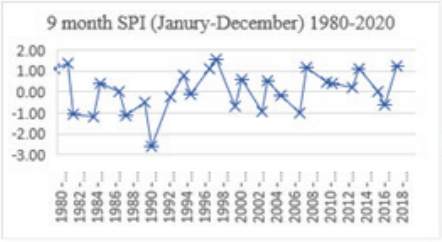

Figure 20: 9 month (January-December) SPI graphics

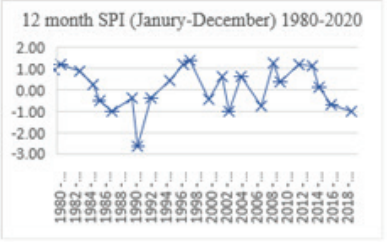

Figure 21: 12 month (January-December) SPI graphics

4. CONCLUSION AND RECOMMENDATIONS

The precipitation values of Izmir station 17220 located in the coastal areas of the Aegean Region of Turkey were adapted to the gamma distribution via the DrinC program and normalized with the standardized precipitation index (SPI) method and drought analysis was performed. The temporal variation of the SPI values obtained by using the 1,3,6,9,12 months serial precipitation data was examined seasonally. According to the results obtained; According to the 1-month SPI index values of Izmir station in whole years 4 season, Spring, summer, autumn and winter seasons, drought class is mostly around normal in the region. From time to time, it is seen that there are seen drought and humid periods. According to the 3-month index values, it was observed that the drought was observed in some periods, and Exceptionally humid period was experienced in 2011, and Extraordinary drought was experienced in 2006. As a result of the calculated 6-month indices values, drought and humid periods were experienced in all years in different terms. As a result of the calculated 9-month indices values, drought periods were classified as Extraordinary drought in 1991 and 2006 and humid periods were experienced as too moist in 1981, 1998 and 2001. As a result of the calculated 12-month indices values, Around normal periods are seen in some years. drought periods were classified as Extraordinary drought, Mild Arid and Medium Arid, and humid periods were classified as Too moist, Moderately humid and slightly

moist. According to the 40-year data used in the study, it has been determined that the drought around normal is constantly observed in the field, and mild arid and moderately arid, severe drought, very severe drought and extraordinary drought classes are experienced from time to time. In humid periods Exceptionally humid, Extremely humid, Too moist, Moderately humid, slightly humid, also experienced from time to time. This is an indication of the importance of water use in the region, especially in agricultural areas. When the drought frequency of the drought SPI values obtained from Izmir meteorology station data is examined, it is observed that there has been an increase and decrease in different periods for 1,3,6,9,and 12 months SPI values. There was significant increase or decrease in other SPI values.

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CHAPTER 9

INVESTIGATION OF THE AMINO ACID CHANGES OF FROZEN-THAWED, BOILED AND FRIED ANCHOVIES MARINATED WITH DIFFERENT FERMENTED FOODS

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INTRODUCTION

The amounts of amino acids change according to the species of fishery products (Özden and Erkan 2011). Many factors impact the amounts of amino acids of fish species, including the region where the fish lives, species, genus, maturity stages, gender, season, diet, and methods. Additionally, processing technologies also affect the amounts of amino acid profiles of fishery products (Erkan et al., 2010a; Erkan et al., 2010b; Özden ve Erkan, 2011; Doğan ve Ertan, 2017). Many studies have also been carried out on the processing of fishery products (Köse et al., 2001; Kılınç, 2009; Inat et al., 2013; Kılınç and Şahin, 2015; Koral, 2016; Kılınç and Sürengil, 2016). Marination is not only an important processing technology of fishery products, but also one of the first processes used to preserve fishery products in ancient times (Kalıştır, 2008). Furthermore, marination is extremely important in terms of being a low-cost technology for increasing the shelf life of fresh fish, softening the muscle structure, and imparting a distinct flavor and aroma to the fish (Kılınç, 2003). The different flavor and aroma of marinated foods are caused by the destruction of proteins and fats. The combined action of acetic acid, salt, enzymes, and bacteria causes these changes in taste and aroma (Mclay, 2001). Cold marinades obtained without heat treatment must be stored in the refrigerator to avoid bacterial spoilage, and to extend the shelf life of the food products (Owens and Mendoza, 1985). Marinades can be prepared in three ways: cooked, fried, and cold. Cold marinades are prepared without the use of heat for frozen, fresh, and salted fish. The aim of the ripening stage, which is the first stage of cold marinades prepared in two stages, is to soften muscle structure of the fish while also ensuring the formation of characteristic flavor (Connell, 1980). The second stage is the rapid placement of fish, which have been removed from the brine solutions, and then packaged with sauce and oil into the plastic containers or glass jars (Gökoğlu, 2002, Kılınç, 2003, Kılınç and Çaklı, 2004). Marinade can be prepared using wine, vinegar, fruit juice, oil, spices, and vegetables etc. (Andres, 1981). It is stated that good quality marinades are expected to be in the 4.0-4.5 pH range. When the pH value is less than 4.5, the bacteria species that cause spoilage and food poisoning can be prevented. Furthermore, tissue cathepsins are very active at these pH levels. Because of the effects of these enzymes, the proteins found in muscles are broken down into amino acids and peptides, resulting in marinade products with their own distinct taste, smell, flavor, and muscle structure (Kılınç, 2003, Kılınç and Çaklı, 2004). Marinades with a limited shelf life may also deteriorate depending on the quality of the fish, brine (concentrations of salt and acetic acid), processing conditions, packaging type, and storage temperature. Microbial reactions, as well as chemical and physical changes in proteins and fats, cause the spoil-

age of these marinared fishery products (Varlık et al., 2007). However, when compared with raw fish, marinating fish can be slowed the increase of unacceptable chemical changes, slow the lipid oxidation, enhanced the sensory characteristics, and extended the product's shelf life throughout cold storage (Sallam et al., 2007). Many studies on marinating fish and other seafood have revealed that it was done using salt and acetic acid, citric acid, gluconic acid, organic acids, and so on. In Türkiye, fish marinades are typically marinated in acetic acid or citric acid, and salt. Then, they are stored in sunflower oil. Because of their high salt content and storage in sunflower oil, these products' negative effects on human health (cardiovascular, blood pressure, and other diseases) limit consumption. With rising consumer demand and awareness for healthier options and natural foods, there is an immediate emergency need for the food industry to create and introduce new food products (Boutheina et al., 2023). To increase the consumption of aquatic products, as well as to develop new healthier alternatives for the marination process, frozen-thawed, boiled, and fried anchovies were marinated with different natural fermented food products (wort, turnip, and kefir). Additionally, the amino acid composition changes of these marinated anchovies were also investigated.

MATERIAL AND PROCESSING

Supply of Anchovy Fish and Removal of Fillets

In the study, 40 kg frozen anchovy fish with an average length of 11±1.0 cm, were obtained as frozen form stored as whole at -18°C from the company in Bornova distrint of Izmir. Anchovies were got in 20 minutes by refrigerated vehicle to the Fish Processing Technology Laboratory of the Faculty of Fisheries of the Aegean University in Bornova District of Izmir in Türkiye without breaking the cold chain. Anchovies, which were provided frozen whole, were separated from their heads, internal organs, and bones after thawing overnight in the refrigerator (+4°C). The anchovies that had been cut into fillets were cleaned with fountain water, and prepared for marinating.

Application of Cooking and Marinating Processes to Anchovy Fillets

In the study, frozen-thawed anchovy fish used as raw materials were marinated in the Processing Technology laboratory located at the Faculty of Fisheries of Ege University. Following the preparation of the anchovy fillets, no cooking was applied (control group), the boiling process was performed to one group for 3 minutes at 170°C and frying in sunflower oil for 3 minutes at 170°C to the other group. The temperature measurement in the

frying and boiling process was carried out with a digital thermometer (Loyka 9263 Plus). Each group was divided into 3 groups and marinade was applied with wort (Rifat Minare, Türkiye), turnip (Doğanay, Türkiye) and kefir (Altınkılıç, Türkiye) for 1 day, 2 days, 3 days at 4°C. The marinating process was carried out at 4°C in glass jars with lids. Fermented products used for marinade were used in a way that was 2:1 (fermented product: fish). Each group was marinated with three different fermented products (wort, turnip and kefir) for three different marinade periods (1 day, 2 days, 3 days). The groups of marination of anchovies are given in Table 1.

Table 1. The groups of marinated anchovies

		Marinated Anchovies		
Marination Days		**Day 1**	**Day 2**	**Day 3**
Frozen-Thawed Marinated	**Turnip**	G2	G3	G4
	Wort	G5	G6	G7
	Kefir	G8	G9	G10
Frozen-Thawed Fried Marinated	**Turnip**	G11	G12	G13
	Wort	G14	G15	G16
	Kefir	G17	G18	G19
Frozen-Thawed Boiled Marinated	**Turnip**	G20	G21	G22
	Wort	G23	G24	G25
	Kefir	G26	G27	G28
Frozen-Thawed Raw Material: G1				

METHODS

AMINO ACID ANALYSIS

Preparation of the samples and the method of amino acid analysis

The samples were weighed according to their concentrations and dissolved in some water. Then, 1 ml of SSA (5-Sulfosalicylic acid dihydrate) was added. In addition, it is completed to 100 ml. The samples were centrifuged at high speed and filtered when necessary and transferred to 1 ml. SSA=2.5 g of 5-Sulfosalicylic acid dihydrate was dissolved in 25 ml of water. 0.2-0.5 g sample (homogenized) was weighed. Then, 5 ml of 6 N HCl was added. 250µl of 2mM phenol was added to prevent oxidation. 0.1 g of Na2SO3 was added to optimize the recovery of cystine, methionine and tyrosine. The samples were hold at 110°C for 24 hours. The pH value of the sample was adjusted to near the neutral (6.7-7.3). In addition, it was complemented by up to 100 ml of water. In addition, centrifugation process was performed for 5 minutes at 4000 rpm. After the filtration pro-

cess, 1 ml was added to the viale. The device method was made using the following application (Agilent Technologies, Santa Clara, CA, USA). It was performed by hydrolysis method in liquid chromatography systems for amino acids. The chemical amino acid analysis were carried out by the Aegean University Central Research Testing and Analysis Laboratory Application and Research Center (EGE MATAL) in Ege University.

STATISTICAL ANALYSIS

In this study, the amino acid values of fishes processing with three different treatments (frozen thawed, boiled fried) and then marinated with three different materials (turnip, wort, kefir) were investigated during three days of storage (1st day, 2nd day, 3rd day). The aim of the study is to determine whether the type of processing, marination type and storage time make a significant difference on the amino acid values considered in this study. In this case, it may be meaningful to visualize the obtained data. One of the techniques used in data visualization is the boxplot. A box plot is a way of looking at a data set to determine its central tendency, spread, skewness and the existence of outliers. This graph contains five summary measures of the distribution of the data: a. The median of the data, b. The lower Quartile (QL), c. The upper quartile (QU), d. The smallest observation, e. The largest observation. The elements of a box plot are shown in Figure 1.

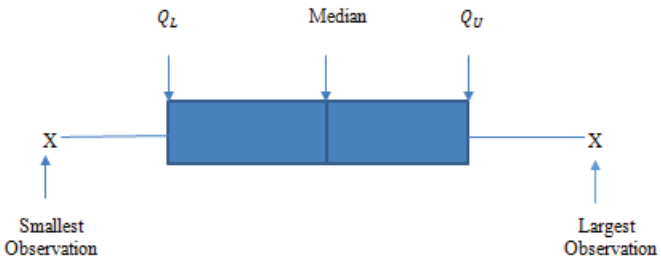


Figure 1: The Box Plot

RESULTS AND DISCUSSION

The amino acid results (g/100g) of frozen-thawed, fried and boiled anchovy marinades are given in Table 2-4.

Table 2. The amino acid results of frozen-thawed and frozen-thawed marinated anchovies (g/100g)

Amino acid g/100g	Frozen-Thawed Marinated Anchovies									
	Frozen-Thawed Raw Material	Turnip			Wort			Kefir		
Groups	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
Aspartic acid	0.337	0.288	0.276	0.287	0.283	0.290	0.294	0.207	0.287	0.319
Glutamic acid	0	0.055	0.707	0.738	0.764	0.736	0.687	0.704	0.724	0.724
Asparagine	0	0.009	0	0	0	0	0	0.001	0.002	0.003
Serine	0	0	0.012	0.010	0.007	0.004	0.006	0.009	0.006	0.004
Glutamine	0	0	0	0	0	0	0	0.006	0.001	0.002
Histidine	0	0	0	0	0	0.003	0.008	0.010	0.006	0.006
Glycine	0	0	0.011	0.008	0.011	0.007	0.008	0	0	0
Threonine	0	0	0.053	0.034	0.038	0.014	0.025	0	0.028	0.029
Arginine	0	0	0.545	0.644	0.589	0.408	0.596	0.750	0.408	0.366
Alanine	0	0	0.029	0.028	0.024	0.015	0.021	0.032	0	0
Tyrosine	0.336	0	0.020	0.018	0.018	0.018	0.020	0.025	0.019	0.016
Cystine	0	0.008	0.011	0.003	0.005	0.003	0.003	0.005	0.004	0.004
Valine	0	0	0	0	0.007	0.020	0.007	0.006	0.006	0.008
Methionine	0	0.016	0.032	0.038	0.029	0	0.034	0.041	0.036	0.045
Norvaline	0	0	0.020	0.018	0	0.017	0.016	0.016	0.003	0
Trptophan	0	0.007	0.003	0.002	0.01	0.001	0.002	0.002	0.002	0.007
Phenylalanine	0	0	0	0.005	0	0.006	0	0.007	0.020	0
Isoleucine	0.034	0.005	0.018	0.015	0.016	0.009	0.012	0.02	0.020	0.019
Leucine	0	0	0.079	0.067	0.013	0	0.007	0.012	0.010	0.009
Lysine	0	0	0.012	0.008	0.009	0.003	0.010	0.015	0.011	0.007
Hdroxyproline	0.233	0	0	0	0	0	0	0	0.042	0
Sarcosine	0.065	0.148	0.114	0.192	0.161	0.114	0.173	0.119	0.129	0.037
Proline	0.040	0.068	0.087	0.074	0.101	0.080	0.082	0.078	0.093	0.075

Table 3. The amino acid results of frozen-thawed fried marinated anchovies (g/100g)

Amino acid g/100g	Frozen-Thawed Fried Marinated Achovies								
	Turnip			Wort			Kefir		
Groups	**G11**	**G12**	**G13**	**G14**	**G15**	**G16**	**G17**	**G18**	**G19**
Aspartic acid	0.272	0.273	0.271	0.310	0.359	0.279	0.282	0.283	0.268
Glutamic acid	0.733	0.701	0.712	0.727	0.647	0.690	0.698	0.696	0.704
Asparagine	0.001	0.001	0.002	0.003	0.006	0.002	0.002	0.001	0.002
Serine	0.003	0.004	0.004	0.006	0	0	0	0	0
Glutamine	0	0	0	0	0	0	0	0	0
Histidine	0.011	0.007	0.007	0,009	0	0.009	0.013	0.010	0.010
Glycine	0	0	0	0	0	0	0	0	0
Threonine	0.023	0.023	0.018	0.031	0	0.023	0.043	0.019	0.033
Arginine	0.384	0.493	0.290	0.228	0	0.738	1.141	0.564	0.856
Alanine	0	0	0	0	0	0.020	0.036	0.019	0.026
Tyrosine	0.016	0.013	0.015	0.014	0.022	0.014	0.015	0.014	0.018
Cystine	0	0.002	0.002	0.002	0	0.002	0.003	0.002	0.003
Valine	0	0	0	0.023	0.070	0.048	0.095	0.040	0.052
Methionine	0.084	0.039	0.032	0.037	0.006	0.020	0.005	0.003	0.004
Norvaline	0.015	0.020	0.003	0.003	0.003	0.005	0.003	0.004	0.003
Trptophan	0	0	0.007	0.001	0.011	0.002	0.002	0.007	0.001
Phenylalanine	0	0	0	0	0	0	0.022	0	0.025
Isoleucine	0.007	0.006	0.007	0.006	0.007	0.006	0.011	0.009	0.010
Leucine	0.006	0.005	0.006	0.013	0.006	0.006	0.008	0.005	0.007
Lysine	0.013	0.008	0.010	0.008	0.008	0.010	0.023	0.007	0.012
Hdroxyproline	0	0	0	0	0	0	0	0	0
Sarcosine	0.140	0.059	0.052	0.060	0.354	0.081	0.134	0.122	0.067
Proline	0.076	0.083	0.087	0.095	0.339	0.080	0.078	0.070	0.088

Table 4. The amino acid results of frozen-thawed boiled marinated anchovies (g/100g)

Amino acid g/100g	Frozen-Thawed Boiled Marinated Anchovies								
	Turnip			Wort			Kefir		
Groups	**G20**	**G21**	**G22**	**G23**	**G24**	**G25**	**G26**	**G27**	**G28**
Aspartic acid	0.253	0.341	0.278	0.268	0.282	0.262	0.272	0.280	0.258
Glutamic acid	0.704	0.673	0.706	0.681	0.677	0.682	0,690	0.693	0.693
Asparagine	0.001	0.003	0.001	0.001	0.001	0.002	0.002	0.001	0.002
Serine	0	0.016	0	0	0	0	0	0	0
Glutamine	0	0	0	0	0	0	0	0	0
Histidine	0.007	0	0.006	0.005	0.006	0.005	0.006	0.007	0.005
Glycine	0	0	0	0	0	0	0	0	0
Threonine	0.019	0.128	0.021	0.017	0.019	0.017	0.020	0.030	0.020
Arginine	0.356	0	0.384	0.267	0.342	0.304	0.458	0.671	0.396
Alanine	0.023	0.004	0.024	0.014	0.012	0.008	0.013	0.018	0.018
Tyrosine	0.012	0.033	0.014	0.013	0.015	0.016	0.015	0.014	0.011
Cystine	0.002	0.003	0.003	0.002	0.002	0.002	0.002	0.002	0.002
Valine	0.029	0.022	0.030	0.025	0.027	0.022	0.035	0.057	0.030
Methionine	0.006	0.023	0.008	0.019	0.021	0.006	0.006	0.006	0.003
Norvaline	0.016	0.005	0.014	0.005	0.003	0.005	0.004	0.003	0.002
Trptophan	0.002	0	0.001	0.001	0.003	0.008	0.007	0.001	0.005
Phenylalanine	0	0	0	0	0	0	0	0	0
Isoleucine	0.006	0.007	0.009	0.003	0.005	0.012	0.005	0.008	0.010
Leucine	0.006	0.005	0.004	0.006	0.006	0.009	0.005	0.006	0.006
Lysine	0.006	0.008	0.003	0.003	0.007	0.006	0.007	0.011	0.004
Hdroxyproline	0	0	0	0	0	0	0	0	0
Sarcosine	0.101	0.095	0.088	0.080	0.142	0.101	0.176	0.142	0.194
Proline	0.092	0.089	0.097	0.083	0.107	0.082	0.096	0.101	0.107

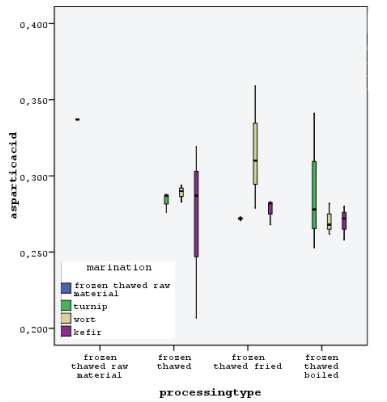
Marinated fish species have significant quantities of substances with biological activity produced during the ripening process with proteolytic enzymes of fish tissue. Multiple technological factors influence the qualitative and quantitative composition of protein hydrolysis products. The impact of key technological factors are very important, including salt and acid concentration in brine, raw material processing, freezing, cover brine

type on the qualitative and quantitative composition of individual nitrogen fractions in flesh and brine during fish marinating (Szymczak et al., 2015). In one research, herrings were marinated in an acetic acid and salt brine. Various nitrogen fractions diffused from fish flesh to brine during marinating, resulted in significant nutritional composition losses of the raw material (Szymczak and Lepczynski, 2016). In another research, the amount of total nitrogen loss from fresh herring fish decreased with an increasing salt or acid concentration, but the amount of total nitrogen loss from frozen herring fish increased with an increasing salt or acid concentration (Szymczak and Kolakowski, 2012). The authors showed in one study that fish products in the Polish market could be an important source of essential amino acids in terms of both quality and quantity. The sulphur-containing essential amino acids and lysine present in fish products could supplement the corresponding deficiency in plant proteins. Nevertheless, it was also suggested that extreme thermal processes, including sterilisation, could have an impact on nutritional composition (Usydus et al., 2009). Another study found that marination accelerated the maximum concentration levels of psychrotrophic bacteria (7-8 log CFU/g) by one logarithmic unit and resulted in five times higher average tyramine concentration levels than the unmarinated product (Jaaskelainen et al., 2023). In contrast to the control sample without protease diffusion, high digestive protease activity in the meat resulted in full ripeness of the marinades. Additionally, the diffusion of digestive proteases during fish cold storage allowed for the production of high sensory quality marinades (Kaminski et al., 2022). Szymczak et al. (2015) reported that fish could be marinated in a salt/acid solution, which were allowed valuable nitrogen fractions to penetrate the flesh. A higher ratio of nitrogen fractions occured during diffusion in flesh and brine. Nitrogen losses from flesh to brine not only could be degraded the quality, yield, and nutritional value of a marinated food product, but they also could be polluted the brine (Szymczak et al., 2015). The author (Ozden, 2005) investigated the changes in the composition of amino acids in the muscle of marinated fish during storage period. It was discovered that threonine, aspartic acid, proline, tyrosine, glycine, and lysine concentrations in marinated products significantly affected the marinated fish quality (Ozden, 2005).

The aminoacid profiles of squid rings were altered by the marination and cooking processes reported by Kılınç et al., (2022). In this study, the marination process increased the concentrations of aspartic acid, asparagine, glycine, norvaline, glutamic acid, histidine, phenylalanine, and tryptophan in squid samples, while decreasing the concentrations of isoleucine, methionine, hydroxyproline, and leucine. Conversely, when compared the cooked squid rings with the cooked marinated squid rings, the marination process caused to increase in the aspartic acid, glutamic acid, sarco-

sine, and proline concentrations, whereas the marination process caused to decrease in the concentrations of histidine, alanine, glutamine, glycine, valine, arginine, cystine, tryptophan, methionine, hydroxyproline, phenylalanine, isoleucine, linolenic, leucine, and palmitic acids (Kılınç et al., 2022). In another study, some of the most common amino acids were found to be lysine, valine, aspartic acid, leucine, and glutamic acid, which varied depending on the preparation and cooking conditions of the food products (Tabak et al, 2021). In the present study aspartic acid, isoleucine, sarcosine and proline were determined in all examined frozen-thawed, boiled and fried marinated anchovies. However, glutamic acid, threonine, arginine, cystine, valine, methionine, norvaline, tryptophan, leucine, lysine were determined in examined samples during marinating process. In frozen-thawed raw material aspartic acid, tyrosine, isoleucine, hdroxyproline, sarcosine, and proline were determined. After the marination of anchovies with turnip, wort, and kefir were done, hdroxyproline was not detected in any of the frozen-thawed, fried and boiled marinated anchovies. However, the values of glutamic acid, threonine, arginine, cystine, methionine, norvaline tryptophan, leucine, and lysine were increased after the marination of all groups. Our findings were consistent with the findings of previous studies (Ozden 2005; Tabak et al. 2021; Kılınç et al., 2022) that the marination and cooking processes influenced changes in some amino acid content of frozen-thawed, boiled, and fried marinated anchovies.

To determine the distribution of amino acid values of marinated frozen-thawed, fried and boiled anchovies with turnip, wort and kefir box plots were used. Box plots were used for the following purposes according to the method of Aczel, (1995): a. to identify the location of a data set based on the median, b. to identify the spread of the data based on the length of the box, c. to identify possible skewness of the distribution of the data set, d. to identify outliers, e. to compare two or more data sets. By drawing a box plot for each data set and displaying the box plots on the same scale, we can compare several data sets. Box plots of each amino acid are given in Figure 2.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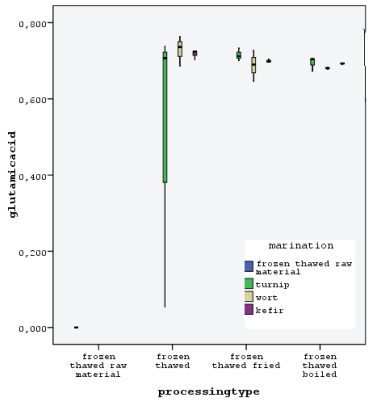
frozen thawed boiled
processingtype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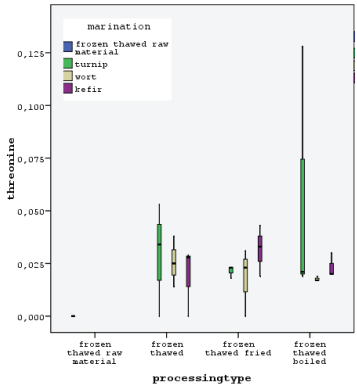
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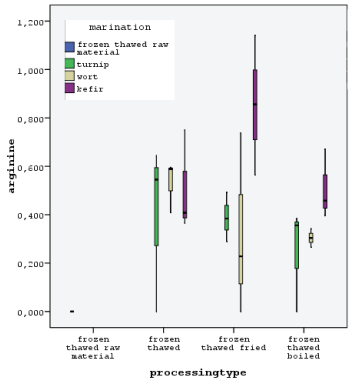
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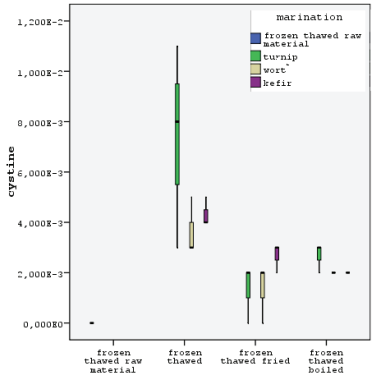
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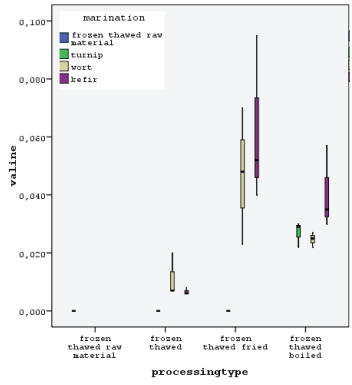
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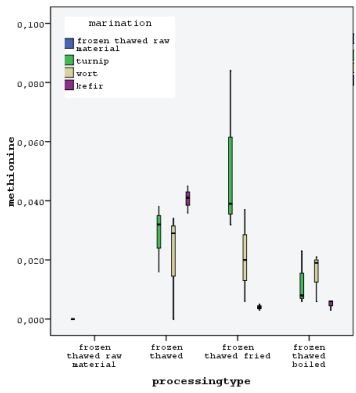
processingtype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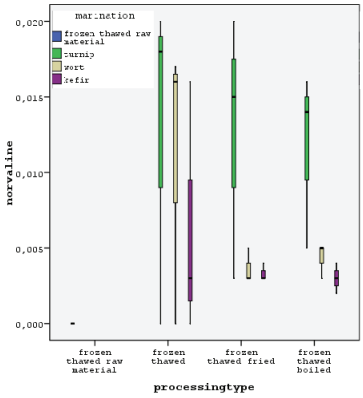
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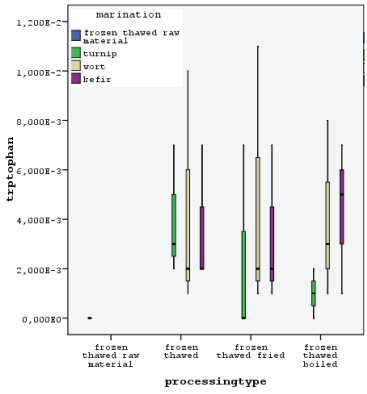
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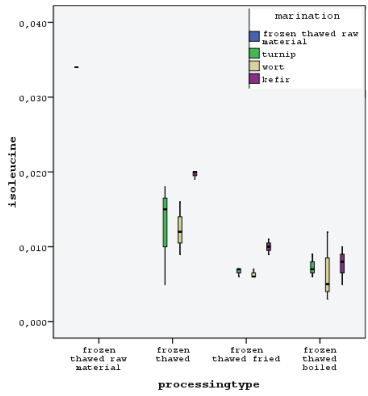
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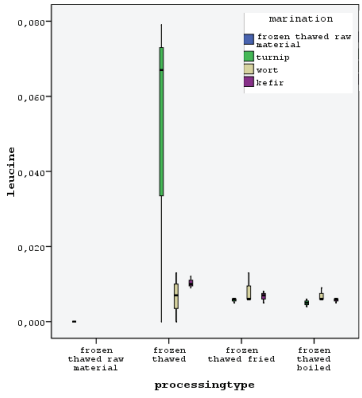
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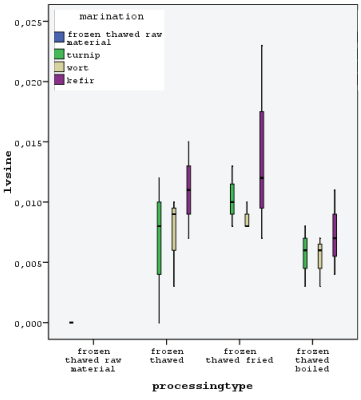
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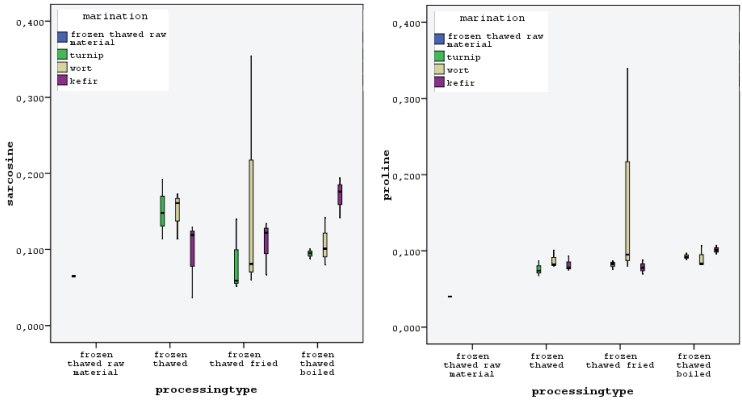

Figure 2: Box plots of the some of the amino acids of marinated anchovies

Generally, the median values in each boxplot were different from each other. Therefore, it could be considered that the means of the subgroups were different. The spread of data in each subgroup was different from others. Therefore, homogeneity was failed in almost subgroups. It was seen that almost all boxes had skewed structures.

Specifically, the spread of kefir in frozen-thawed group, wort in frozen-thawed fried group and turnip in frozen-thawed boiled group was different from others in the boxplot of aspartic acid. More homogeneous structure was seen except the turnip in the frozen-thawed group in the boxplot of glutamic acid. It was seen that there were non-homogeneous structures both in subgroups and between groups in box plots of both threonine and arginine. For cysteine: Other than turnip in the frozen-thawed group the spread of data was less. Nonhomogeneous structures were seen for valine and methionine. The spread of data was wide for norvaline and trptophan. The spread of data was so narrow in frozen-thawed fried group for isoleucine. More homogeneous structure was seen except the turnip in the frozen-thawed group in the boxplot of leucine acid. Similarly, homogeneous structure was seen except the wort in the frozen-thawed fried group in the boxplot of proline. Different medians and different structures were seen in all subgroups of sarcosine and lysine amino acids.

From the box plots, it is seen that the subgroups are not homogeneous, that is, the group variances are different and generally skewed distributions occurs. These assumptions are the assumptions of the ANOVA test and it is understood that these assumptions are failed. The accuracy of these results which are visually obtained from the box plots can be checked with hypothesis testing. The normality assumption was checked using the Kolmogorov-Smirnov test, while the homogeneity assumption was checked using the Levene test for the ANOVA. However, these two assumptions were not

met in most subgroups. For this reason, the significance of the difference between groups was checked using the Kruskal Wallis and Mann Whitney nonparametric tests (Montgomery and Runger, 2003, Panic 2005).

The Kruskal Wallis test was performed for aspartic acid, glutamic acid, threonine, arginine, cystine, valine, methionine, norvaline, trptophan, isoleucine, leucine, lvsine, sarcosine, and proline according to the processing type. According to the Kruskal Wallis test, it was determined that the glutamic acid (*p-value*=0,037), cystine (*p-value*=0,000), valine (*p-value*=0,014), isoleucine (*p-value*=0,008), lvsine (*p-value*=0,029) and proline (*p-value*=0,026) values showed differences statistically significant depending on the type of processing. The Mann Whitney test was used to determine which processing type caused these differences. According to the Mann Whitney test, the difference between frozen – thawed and frozen – thawed fried process types was found to be significant for cystine (*p-value*=0,000), and isoleucine (*p-value*=0,008) amino acid values. Similarly, the difference between frozen – thawed and frozen – thawed boiled process types was found to be significant for glutamic acid (*p-value*=0,024), cystine (*p-value*=0,000), valine (*p-value*=0,000), isoleucine (*p-value*=0,006), and proline (*p-value*=0,014) amino acid values. Moreover, the difference between frozen–thawed fried and frozen–thawed boiled process types was found to be significant for lysine (*p-value*=0,004) and proline (*p-value*=0,05) amino acid values.

The Kruskal Wallis test was also performed for aspartic acid, glutamic acid, threonine, arginine, cystine, valine, methionine, norvaline, trptophan, isoleucine, leucine, lysine, sarcosine, and proline according to the marination type. According to the results, it was determined that arginine (*p-value*=0,047) and valine (*p-value*=0,018) values showed differences statistically significant depending on the type of marination. According to the Mann Whitney test, the difference between turnip and wort was found to be significant for only valine (*p-value*=0,040) values. The difference between turnip and kefir marination types was found to be significant for both arginine (*p-value*=0,024) and valine (*p-value*=0,004) amino acid values. The difference between wort and kefir marination types was found to be significant for only arginine (*p-value*=0,05). Again, the Kruskal Wallis test was performed for the aspartic acid, glutamic acid, threonine, arginine, cystine, valine, methionine, norvaline, trptophan, isoleucine, leucine, lysine, sarcosine, and proline according to the time. However, no significant results were obtained for any amino acid according to time. In other words, the amino acid values did not show a significant difference according to time.

CONCLUSION

Aspartic acid, tyrosine, isoleucine, hdroxyproline, sarcosine, and proline were measured in frozen-thawed raw material (G1), whereas aspartic acid,

isoleucine, sarcosine, and proline levels were measured in all frozen-thawed (G2-G10), fried (G11-19), and boiled (G20-28) marinated anchovies tested in this study. However, hdroxyproline was not detected in any of the frozen-thawed, fried, and boiled marinated anchovies (G2-G28) after the marination with turnip, wort, or kefir except for frozen-thawed raw material (G1). Additionally, glutamic acid, threonine, arginine, cystine, valine, methionine, norvaline, tryptophan, leucine, and lysine were found in the marinated samples. Morever, after the marination, all groups (G2-G28) of glutamic acid, threonine, arginine, cystine, methionine, norvaline trptophan, leucine, and lysine levels increased. The Kruskal Wallis test was performed for determining the aspartic acid, glutamic acid, threonine, arginine, cystine, valine, methionine, norvaline, trptophan, isoleucine, leucine, lysine, sarcosine, and proline according to the time. However, no significant results were obtained for any amino acid according to time. The Kruskal Wallis test was also performed for aspartic acid, glutamic acid, threonine, arginine, cystine, valine, methionine, norvaline, trptophan, isoleucine, leucine, lysine, sarcosine, and proline according to the marination type. According to the results, it was determined that arginine and valine values showed differences statistically significant depending on the type of marination. According to the Mann Whitney test, the difference between turnip and wort was found to be significant for only valine values. The difference between turnip and kefir marination types was found to be significant for both arginine and valine amino acid values. The difference between wort and kefir marination types was found to be significant for only arginine. According to the Kruskal Wallis test, it was determined that the glutamic acid, cystine, valine, isoleucine, lysine and proline values showed differences statistically significant depending on the type of processing. The Mann Whitney test was used to determine which processing type caused these differences. According to the Mann Whitney test, the difference between frozen–thawed and frozen–thawed fried process types was found to be significant for cystine, and isoleucine amino acid values. Similarly, the difference between frozen–thawed and frozen–thawed boiled process types was found to be significant for glutamic acid, cystine, valine, isoleucine, and proline amino acid values. Moreover, the difference between frozen–thawed fried and frozen–thawed boiled process types was found to be significant for lysine and proline amino acid values.

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