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UNIVERSIDADE DE TRÁS-OS-MONTES E ALTO DOURO



UNIVERSITY OF CRAIOVA FACULTY OF AGRONOMY

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HEALTH EFFECT OF PLANT BASED BIOACTIVE COMPOUNDS

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Abstract

Phytochemicals naturally occurring in plants and biologically active are chemical compounds. In addition to serving as a natural defense system in plants. They are also responsible for color, aroma and taste. Food provides essential nutrients for life and phytochemicals necessary for our bodies to promote health and prevent disease. Cardiovascular disease, cancer and obesity are ranked as the first and second leading causes of death in world. Regular consumption of fruit and vegetables reduces the risk of most diseases. Functional foods that contain significant amounts of bioactive components may provide desirable health benefits beyond basic nutrition. It is estimated that more than 5000 phytochemicals have been identified, but a large percentage still remain unknown. However, more and more convincing evidence suggests that the benefits of phytochemicals in fruit and vegetables may be even greater than is currently understood because oxidative stress induced by free radicals is involved in the etiology of a wide range of chronic diseases.

Key words: *phochemicals, phenolics, antioxidants, fruit, vegetables.*

INTRODUCTION

Increasing interest in natural products and increasing awareness of healthy nutrition in people therefore, studies on the extraction of bioactive compounds are carried out. All studies in the field of medicine, from vegetables, fruits, grains, cereals and legumes heart diseases of eating a rich diet, such as hypertension, cancer, and diabetes risks that have besieged its population significantly It is believed that it can reduce (Wargovich, 1997). Phytochemicals naturally occurring in plants and biologically active are chemical compounds. In addition to serving as a natural defense system in plants. They are also responsible for color, aroma and taste. It is estimated that more than 5000 phytochemicals have been identified, but a large percentage still remain unknown (Shaidi and Naczk, 1995). Phytochemicals are general nitrogen-containing, alkaloids, carotenoids, organosulfuric, and phenolic compounds (Savaş, 2010). These compounds used against many diseases today. In recent years,

phytochemicals have been considered due to being their protective and preventive effects against some kinds of cancer and heart diseases. The most important effect of these compounds on health is to be functional like antioxidants that reacted with the free oxygen molecules and free radicals in the body. Phytochemicals had the feature chelating metal ions. Phytochemicals perform duty as antiallergenic, an anti-inflammatory, antimicrobial, antithrombotic, anticancer, antiatherogen, antiulcer and vasodilator agent (Demir and Akpınar, 2020). Fruit and vegetables contain a wide variety of antioxidant compounds (phytochemicals) such as phenolics and carotenoids that may help protect cellular systems from oxidative damage and lower the risk of chronic diseases (Liu, 2003). This in the study; widely used nowadays from the biological activities of natural phytochemicals; antioxidant, antimicrobial, antifungal, antidiabetic, its anti-inflammatory,

anticancer and antihypertensive properties and studies on these issues have been compiled.

Phenolic Compounds

These compounds are a benzene ring with one or more hydroxyl groups attached, and produced by plants with functional groups as secondary metabolites. The amounts of these compounds vary according to plants and are the most abundant in plants. More than 30,000 phenolic compounds are produced by plants as a defense mechanism in nature available. We get 5,000 to 10,000 of these phenolic compounds from food on a daily basis (Manach, 2004). They can be classified in different ways that range from simple molecules to highly polymerized compounds. Based on their carbon skeleton, phenolic compounds are classified in non-flavonoids (phenol, phenolic acids, benzoquinones, stilbenes and lignans groups) and flavonoids (flavonols, flavones, anthocyanins, flavanones, isoflavones and flavan-3-ols) compounds (Demir, 2020). The growing interest in this class is due to their antioxidant potential and the association between their consumption and prevention of some diseases. The health benefits of these chemical compounds are directly linked to a regular intake and their bioavailability. Phenolic compounds are described as the natural source of the antioxidant need for metabolism; they demonstrate antioxidant activities by binding free radicals or forming chelates with metals. In addition to their antioxidant properties; they also have antiglycemic, anti-allergenic, anti-allergenic, anticarcinogenic, antimicrobial, anticholesterol, anti-inflammatory, antithrombotic, vasodilator; they are used in cosmetics, medicines and food industries (Güzel & Akpınar, 2019; Naczek & Shahidi, 2006; Pehlivan et al., 2018). The number of OH groups in the phenol ring in which they have these effects increases as they increase. Phenolic compounds have the ability to delay, slow or prevent oxidation at low

concentrations and to remain in a stable form when they become free radicals (Silva et al., 2005). Phenolic compounds increase the activity of enzymes responsible for inhibition of cancer cells, preventing the development of nitroamine, which plays an important role in tumor formation and cancer formation. It also regulates the ionic equilibrium and the pH in the intestinal flora. Intracellulars are responsible for preserving the integrity of the matrices, allowing the cell to be resistant to environmental effects (Güney et al., 2008).

Flavonoids

Flavonoids are secondary metabolites with significant levels of antioxidant activity and chelating properties, and are not synthesized by humans. More than 4,000 flavonoids have been identified in nature. They are named after the ring structures such as anthocyanides, flavons and flavonols, flavanones, flavanols (catechins) and isoflavones (Sivam et al., 2010). More than 1,500 flavonoids are available. Flavones (e.g. flavonoids of apigene found in daisies), Flavonoids (e.g.; corstice found in grapefruit, rutin found in black wheat, ginkgoflavon glycosides found in ginkgoflavon) Flavonones (e.g. hesperidine found in orange fruit, silibin found in the camel). Flavonoid compounds have bioactive activities such as antioxidant, anti-inflammatory, anti-allergic, antiviral, anti-aging, and anticarcinogen (cytotoxic) (Çağlar et al., 2017). Flavonoids have biological activity against allergies, inflammation, free radicals, hepatotoxins, microbes, viruses, ulcers, tumors, and platelet clustering. Flavonoids also inhibit specific enzymes: for example, flavonoids inhibit the angiotensin-converting enzyme (ACE) that increases blood pressure: they do not splinter the cyclooxygenase enzyme that breaks down prostaglandins and accumulate platelets' adhesiveness by reducing the adhesion. Flavonoids also protect the vascular system and strengthen the capillaries that carry oxygen and

essential nutrients to all cells (Özcan et.al., 2003). In addition, flavonoids reduce the risk of estrogen-induced cancer by blocking estrogen-producing enzymes. Flavonoids can also achieve this effect by blocking the estrogen synthesis enzyme that binds estrogen to receptors in various organs. Generally, onions, apples and cabbage are abundant (Fidan&Dündar, 2007).

Terpenes

Terpenes found in vegetables, soy products, and grains are one of the largest classes in phytochemicals; as can be seen from recent studies on β -caroten, the most densely working terpenes are carotenoids. Terpenes serve as antioxidants in fat, blood, and other body fluids. They form the most important compound group of volatile oils. Terpenoids are very common in plants, just as they do in human cells, they protect plants from reactive oxygen (Özcan et.al., 2003).

Carotenoids

This class includes live substances found in foods such as tomatoes, parsley, oranges, grapefruit, spinach, and red palm oil. Carotenoids that give color to egg yolk protect the unsaturated fatty acids found in egg yolk. There are more than 600 naturally occurring carotenoids, often thought to be precursors of vitamin A. However, less than 10% of those with vitamin A activity. Within carotens, only α , β , and E-carotene A activity is shown. Beta carotene is the most active of these. Alpha carotene has as much antioxidant activity as 50-54% of beta caroten and epsilon carotene as 42-50% of beta carotene as antioxidant activity. Along with the carotens mentioned above, carotens such as gamma carotene, lycopene and lutein are thought to be protective against cancers of the lung, colon, breast, uterus, and prostate (Bendich and Olson, 1989). The protective properties of carotens are unique to tissues. Therefore, carotens have more protective effect when consumed together.

Carotens strengthen the immune system and protect skin cells from UV radiation. In addition, carotens allow safe disposal of waste materials and toxins from the body by disseminating the phase II enzymes of glutathione found in the liver (Özcan et.al., 2003).

Isoflavones

Isoflavones are found in the subclass of phenols in beans and other beans, and are slightly similar. Isoflavones, like flavonoids, block enzymes that increase their development to tumor flavonoids. Genistein and daidzein are the most isoflavones found in soy products and in *Pueraria lobata* (kudzu). Breast cancers are rarely found in people who are known to diet rich in soy products. *Pueraria* has gained importance for people who consume alcohol, because the phytochemicals found in this plant and prostate are thought to alter the activity of alcohol detoxification enzymes, which is the rate of alcohol dehydrogenase converting alcohol into aldehydes. In the end, alcohol tolerance decreases and the desire for alcohol decreases (Ozcan et.al.,)

Indols

Indols are found in this subclass of phytochemicals that interact with vitamin C. That's not surprising, because vegetables containing indole also contain significant amounts of vitamin C, and indole complexes bind chemical carcinogens in the stomach intestinal system and activate detoxification enzymes, and the biotransformation products of indoles are produced by the influence of stomach acid. The most active product is "ascorbigen," thought to be a vitamin C.

Coumarins

Coumarins are heterocyclic compounds consisting of enriched benzene and 2-pyrone rings in various plants such as tonka beans. Their derivatives exhibit a wide variety of bioactive properties such as antioxidants, anticoagulants,

antibacterials, antiinflammatory, antitumoral, antivirals and enzyme inhibition (Li et.al, 2020).

Lycopene

High licopen intake or high serum concentrations have shown a significant reduction in the risk of stroke and cardiovascular disease. Positive effects of tomatoes and licopen reinforcement have also been observed on blood lipids (ldl-cholesterol), blood pressure (systolic blood pressure) and endothelial function (flow mediated expansion). Licopen also relieves oxidative stress by reducing the length of the DNA tail. At least 85% of our dietary lycopene comes from tomato fruit and tomato-based products, the remainder being obtained from watermelon, pink grapefruit, guava and papaya (Khan et.al., 2021).

Tocopherols and Tocotrienols

are also found naturally in grains and in palm oil, similar to tokoferols and tokoferols. Tocotrienols are thought to stop the development of breast cancer cells. However, tocoferols do not have this effect. Researchers have observed that there is no correlation between tokoferols and tocotrienols' biological functions. The most cholesterol-reducing effects of tocotrienols have been studied (Szewczyk et.al., 2021).

Tannens

Tannens have a variety of effects on biological systems because they have metal ion chlation, protein breakdown properties. When exposed to heat at a certain interval, these compounds become pyrogalic acids. Pyrogalic acid has been shown to have an anticarcinogenic effect by mutating DNA. Particles have also been reported to decrease serum lipid levels and have a regulating effect on insulin release (Hagermann,2012).

Sulphides

Glutation transferase increases the effectiveness of detoxification enzymes such as NADPH and quinon reductase. It has also been shown to play

a role in the removal from the body of metabolites such as NADPH and quinon reductase, which also contribute to antioxidant defense. It has been isolated at significant levels from garlic, onions, onions, porridge, pineapple and broccoli (Cao et.al., 2014).

Isothiocyanates

The most commonly known effect is to prevent DNA damage. This property is thought to be achieved by directing enzymatic activities; isothiocyanates are found at significant levels in sources such as water terrace, radish, and cabbage.

Lignans

Lignans are formed by the oxidative dimersion of two phenylpropane units. Interest in lignans and their synthetic derivatives increases due to their potential applicability in cancer treatment and in various other pharmacological areas. Anthriscus sylvestri (L.) has reported high levels of lignan compounds in the Hoffm plant to be cytotoxic to cancer cells, as well as antiviral, anti-inflammatory and antiallergic properties.

Stylbens

Stylbens are a polyphenol group that is very common in plants. Its most known compound is resveratrol (3.5.4'-trihydroxystilben) '. Resveratrol is found in red wine, as well as in the outer shell of grapes. Stylbens are produced by plants in response to pathogens or various stress-related infections; grapes, strawberries and peanuts have been detected in more than 70 species of plants, mainly in grapes, strawberries and peanuts. A study found stilbens isolated from red wine to function as radical vacuum cleaners, and that stilbens have a significant antioxidant effect. It was also reported that stilbens have a significant antioxidant effect (Vincenzi et.al.,2016).

Cathechin

The chemical structure of catechin is slightly different from other flavonoids. However, they

have the same protective properties. The most common catechins are the Gallic esters of epicatechin (EC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). These are all green teas found in *Camellia sinensis*, and the protective benefits of green tea are thought to derive from these compounds.

Glucosinolates

Glucosinolates found in vegetables belonging to mustards are powerful activators of liver detoxification enzymes. They also regulate white blood cells and cytokines, diethylations and sulphane are bio-transformation products of glucosinolates. Each of these protects specific tissues, especially in the chest, liver, colon, lung, stomach, and esophagus, is a function of these compounds to block enzymes that support the development of tumors in the chest, liver, colon, lung, stomach and esophagus (Yılmaz & Demirel, 2012).

Anticyanides

Anticyanides are a highly noticeable group of flavonoids. Technically known as "flavonols," these compounds cross-bind or form "bridges" that bind and strengthen the cross-threads of collagen proteins. Collagen is the most found protein in our body that forms the soft tissue, tendons, ligaments, and bone matrix. The water-soluble anticyanides remove the free radicals they encounter in tissue fluids. This is particularly beneficial for athletes and other active people, because large amounts of free radicals occur as a result of high bodily activity (Koca et.al., 2006).

RESULTS AND DISCUSSIONS

The bioactive properties of phytochemicals of human health importance have been studied in many studies and are still being studied. Instead of the antioxidant properties of these compounds, inhibiting the development of antimicrobial and pathogenic microorganisms, balancing blood glucose levels with antidiabetic

properties, inflammatory properties, inflammatory properties, anticancer properties, anticancer properties to treat cancer and reduce the risk of cancer, to control blood pressure with its antihypertensive properties, to prevent the harmful effects of free radicals that may occur in the later stages of life, and thus to enable people to live a healthier life has been shown in studies. Phytochemical diet is seen to play an important role in reducing diseases caused by today's lifestyle, and to respond to consumers' desire to consume healthier products.

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THE EFFECT OF PRIMING PROCESSING ON THE ROOT AND SEEDLING DEVELOPMENT OF CHICKPEA SEEDS

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Abstract

Today, the cultivation area allocated for edible grain legume varieties, which cannot meet expectations due to drought, is decreasing over time. A faster and more efficient development period should be provided in order for the cultivation area of legumes to increase and for legume production to become preferable by the producer.

At the same time, with the completion of the production process in a shorter time, both more production diversity will be provided and planting traffic will be minimized.

Priming is defined as all controlled processes that are carried out on the seed before planting, initiating the metabolic activity necessary for germination, but not allowing root exit.

In order to bring the legume production back to the producer and make it more attractive, to provide rapid production in terms of germination and seedling development, and at the same time to increase the amount of input, pre-treatment (priming) applications will be made to the seeds, which are the production material.

Although it varies according to the forms applied as a result of KNO₃ priming processes, germination speed and strength will increase, progress will be made in seedling growth and development, yield increase will be achieved and weed damage will decrease as production time will decrease. Improvement will be achieved for the manufacturer and will increase the preferability.

At the same time, durable plants will be obtained and production will be tried to be completed with minimal problems. In the research conducted for this purpose; When examined statistically in terms of root count, root length, root weight, seedling height and seedling weight characteristics, 1% KNO₃ dose ranked 1st. We recommend that our producers use a 1% dose of KNO₃ as a suitable priming concentrate in chickpea production.

Key words: Seed priming, Chickpea, Yield, KNO₃ dose

INTRODUCTION

When the chickpea species under the heading of edible grain legumes is examined in itself, although it has different climate requests, it is tried to be produced within the grain / legume planting seizure system in the regions where it grows in line with its wishes. Due to these planting seizures, it is important to research and apply economical and sustainable production methods that will ensure the high germination power, germination rate, rapid seedling

development and growth, high yield and quality product of edible grain legume plants grown in the appropriate time interval (Akers and S.W., 1990).

Profitability and sustainability in production are achieved not only by increasing yields, but also by reducing the elements of expenses (irrigation, fuel, time, fertilizer, medicine). While performing the production, it is important for the profitability and sustainability of the production

method used to warn the seed for germination, to increase the seed germination rate, to reduce soil traffic by shortening the germination time, to complete the plant faster, stronger and to complete its development in a time frame lower than expected and to reduce the use of fertilizers and chemical drugs (herbicides, fungicides, etc.) (Bradford and K.J., 1995).

The need for edible grain legumes in our country is increasing day by day due to the fact that the cost is more than the input as a result of global warming, loss of soil wealth and low yield. In

line with the pre-treatments to be applied due to the low yield encountered due to the environmental conditions, it is important to improve the germination prevention or retardant effects of the conditions such as hard shelling and cracking in the seed and at the same time the production time (Elkoca, Ertaş, 2007).

In addition, it is important to reduce the germination-promoting fertilizer forms required for high and quality production in crop production and to contribute to cost reduction.

Weed controls are carried out based on weed exit times along with plant growth.

Due to the pre-treatments applied, the plant will finish its development in a shorter time and there will be a decrease in the chemical form and dose needed as a result of completing it before the predicted weed exit time.

It will be tried to reveal the effect of the pre-treatments applied in the cultivation of edible grain legumes on the germination speed and strength of the plants, as well as the elements of seedling development and growth and to reveal the pre-production method necessary for the producers to make an economical and sustainable cultivation (Capron et. al., 2000).

In summary; With the increase in the application of priming process, significant progress has been made in eliminating the deficiency in seasonal transitions or in the market without much interruption and at the same time there will be reductions in expenses during the growing period.

MATERIAL AND METHOD

The trial consists of two different stages. The first stage; 100 ml pure water (control) and KNO_3 mixtures with 1%, 2% and 3% concentrate were prepared (Figure 2), the seeds were set to 4 layers and kept in pure water and mixtures for 18 hours.

The second stage is; At the end of 18 hours, seeds were sown in the violas (Figure 4).

Our trial was carried out in laboratory environments by providing appropriate and equal temperature values.

This study was carried out at Ege University Faculty of Agriculture, Department of Field Crops, Cytogenetics Laboratories.

Yellow Chickpea variety seeds belonging to *Cicer arietinum* species were used as production material. After the viola, peat soil and seed preparations (Figure 3). were made for seed sowing, seed sowing was carried out in the viola. Seed supply was provided from Değirmen Tohumculuk in İzmir Konak.

KNO_3 osmotic solution was used in chickpea priming applications. 3+1 repetition has been made; The seeds were soaked for 18 hours in a 1%, 2% and 3% KNO_3 osmotic solution, 50 each. The repetition of control is excluded from this process.

At the end of 18 hours, the seeds were taken directly to the violas and sown in the peat soil. After planting, they went to the laboratory and watered the chickpeas with pure water according to the request of the soil (Figure 5).

After the trial is established, the number of germinated seeds will be counted on the 6th and 14th day from sowing and it is noted that the germinated seeds are compared between the recurrences.

Seed sowing was done on 23.11.2021 (Figure 4) germination was observed for the first time on 28.11.2021 (Figure 1).

Related Images

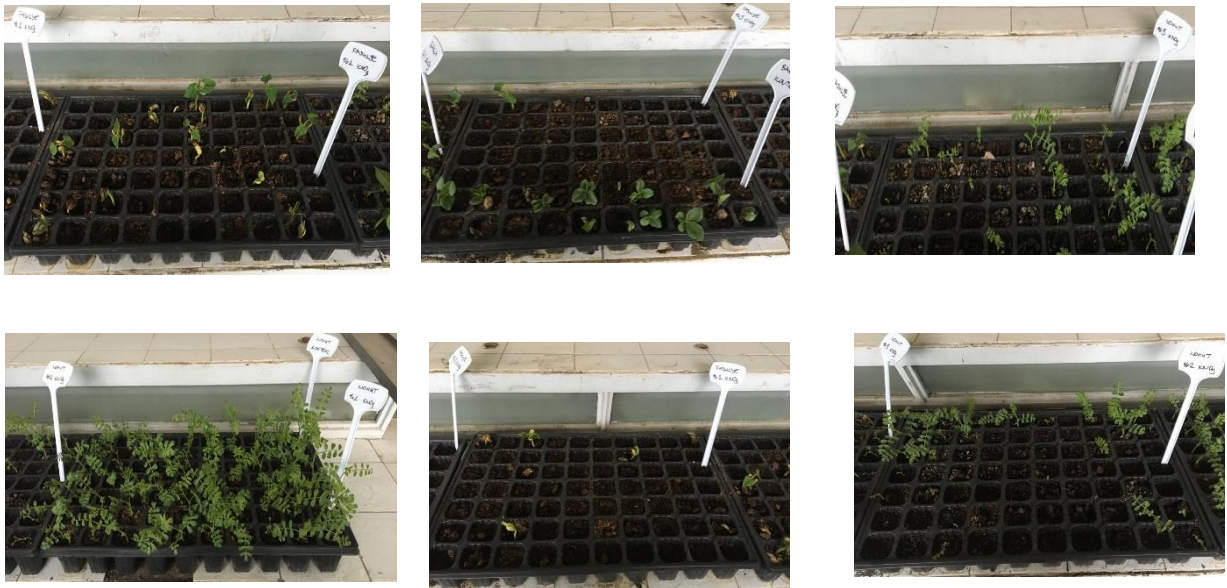


Figure 1: Germination rates



KNO₃ Solution Preparation



Figure 2 Soil Preparation



Figure 3 Seed Sowing



Figure 4

Watering



Figure 5

RESULT

In chickpeas; When examined statistically in terms of the characteristics of the development of roots and seedlings, it ranked 1st. We recommend that our producers use a dose of 1% KNO₃ as a suitable priming concentrate in chickpea production.

RESOURCES

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POLYPLOIDY BREEDING APPROACHES ABOUT IMPORTANT NATURAL SWEETENER STEVIA

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Abstract

Due to the rapidly increasing obesity, diabetes and other diseases around the world, the search for healthy and natural sweeteners is increasing rapidly. Stevia (*Stevia rebaudiana*Bertoni) has the potential to be a significant alternative food sweetener with its diterpene glycosides content, which has a much higher taste than sucrose. However, the breeding and production of stevia has quite difficulties due to the perennial nature of the plant, self-incompatibility/ cross pollination structure and low germination potential. It is needed to use traditional breeding methods and biotechnological breeding approaches together for scientific studies on crop improvement and steviol glycosides production in stevia. The main methods used in stevia breeding are selection (especially recurrent selection), mutation, synthetic breeding, repetitive selection, and polyploidy breeding. Polyploidy breeding is quite valuable technique as they have larger leaves and higher steviol glycoside potential than diploids. This article shows the importance of polyploidy in stevia breeding and reviews all significant data accessible in previous scientific studies about stevia polyploidy breeding.

Key words: Sweetener, *Stevia rebaudiana*, Biotechnological, Polyploidy

INTRODUCTION

Stevia is a perennial, herbaceous plant belonging to the Asteraceae family, has a chromosome number of $2n=22$, and is an important short-day plant due to the sweetening compounds it contains. Stevia climatic conditions are subtropical regions with an average temperature of 25°C, semi-humidity, and precipitation between 1500-1800 mm per year (Yadav 2011, Maheshwer 2005). It naturally grows at an altitude of 200-500 m (Singh and Rao, 2005). Stevia originated in the mountainous regions in the northeastern part of Paraguay (on the border with Brazil) in South America. The local

people here have been using stevia for centuries in food and medicine.

Stevia rebaudiana (Bertoni) is a valuable natural sweetener, 300 times sweeter than sucrose thanks to its diterpene glycoside contents and it has zero calorie. (Yao et al., 1999). Stevia is alternative to the synthetic sweeteners that are now available to the diet conscious consumers. Stevia is safe for people because the sweet compounds pass through the digestive process without chemically breaking down and so, blood sugar level can be controlled (Strauss 1995). Eight types of diterpene glycosides were identified in stevia leaves. There are 4

important sweeteners in these glycosides. These are stevioside, rebaudioside A, rebaudioside C, and dulcoside A, which are 210, 242, and 30 times sweeter than sucrose, respectively. Rebaudioside A, which is produced in the leaves and stems of stevia, is one of the most interesting glycosides. The reason for this is that steviosides leave a bitter after taste and rebaudioside A does not leave such an aftertaste. Rebaudioside A has significant potential to be used as an alternative to other synthetic sweeteners as a substitute for sucrose (about 300 times more sweetener) (Rondi, 1980). Stevia leaves can be directly added to the beverages or could be added that is in the powder form. A liquid form of stevia is also available that could be added in the beverages. In addition to being naturally obtained and reliable, another important advantage of stevia over other artificial sweeteners is that it offers the opportunity to be boiled in the food field and used in foods cooked at high temperatures due to its high structure and pH stability (İnanç and Çınar, 2009).

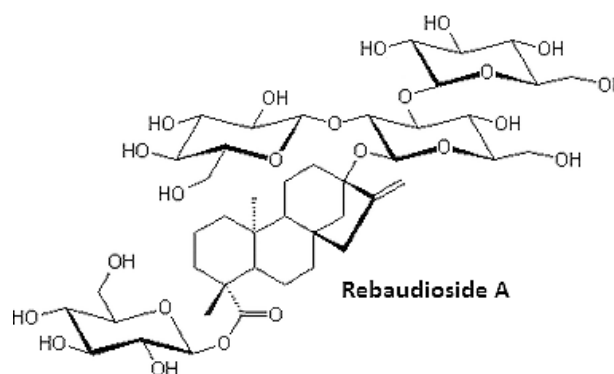


Figure 1. The chemical structures of rebaudioside A

In addition to its sweetening feature, stevia also has medicinal value and usage. These include antihyperglycemic, anticarcinogenic, antibacterial, antifungal and antiallergic properties (Jeppensen 2002, 2003; Rajas and Miranda, 2002; Chan et al., 1998). In addition to this, it also has a birth control feature and

a preventive feature against tooth decay (Melis, 1999; Lemus-Mondaca, 2012).

As a result of studies on stevia and human health, the American Food and Drug Administration (US FDA) decided in 2008 that rebaudioside should be generally recognized as safe (Herranz-Lopez et al., 2011). In Turkey, steviol glycosides can be used as sweeteners according to the Turkish Food Codex Regulation on Food Additives (Anonymous, 2015).

The global stevia market reached a value of US\$ 590 Million in 2020. Looking forward, IMARC Group expects the market to grow at a CAGR of 8.6% during 2021-2026. The cost for Stevia Rebaudiana of 1kg is around \$90-\$100. The great challenge in the stevia rebaudiana market is its high price. Its prices are relatively high compared to sugar.

Stevia Breeding

Despite the potential market size of stevia, agricultural production of this crop still problematic and insufficient to meet growing global demand. Research and initiatives on the production and breeding of stevia are still quite limited.

Main reasons why stevia yields are still low and unstable in many countries are the absence of well adapted and available varieties, high input costs limited expertise in the cultivation, insufficient disease management and poor irrigation.

Stevia researches still depend on a sustainable agricultural practice that takes into account high leaf yield, high leaf-to-stem ratio, high steviol glycoside content capacity, high adaptability to various environmental conditions, and high germination rate of seeds, respectively.

The most important goals in stevia breeding are to obtain higher performing and yielding cultivars with high glycosides. While the glycoside ratio in the dry matter of stevia

leaves is between 2 and 10 percent, this ratio has increased to 20 percent as a result of 30 years of stevia breeding (Brandle and Rosa, 1992). Also, resistance to abiotic and biotic stresses are other important characteristics for stevia breeders (Tavarini et al., 2018).

Most stevia cultivars show as morphologically different features from one another which means adequate amount of phenotypic diversity available for breeding (Othman et al., 2015).

Stevia can be propagated by seeds, vegetative cuttings and tissue culture. Stevia has sporophytic self-incompatibility nature (Raina et al., 2013). This self-incompatibility causes insufficient germination percent and low seeds yield which are the major problems in stevia propagation. Stevia quality, quantity and growth of steviol glycosides and the ratio of the total stevioside and Reb. A are affected by seeds low germination, viability and fertilization directly. That means its agriculture from the seeds is not effective. Modern propagation techniques such as in vitro regeneration and/or tissue culture are important approaches to increase the production of this important species.



Figure 2. Stevia Leaves (A) and Flower Buds (B)

Breeders apply genetic and classical breeding methods that contribute to the improvement of stevia. Some of them are traditional breeding methods of stevia such as selection for the traits with molecular research, i.e., polyploidization, selective markers joint determination with exact traits, or essential

interference in the plant genome to constructing a transgenic diversity.

it is essential to understand the inheritance and basic natures of a given trait to improve stevia traits. Also, metabolic pathway analysis, enzymes determination of those paths, genes regulation approaches accountable for the procedures could provide important information to development of desired characters (Yadav and Guleria, 2012). For stevia breeding efficiency, the genetic inheritance of glycosides is crucial. For the steviol glycoside pathways, some of the responsible genes were identified (Brandle et al., 2002). As genetically, steviosid and rebA are negatively correlated while rebA and rebC are positively correlated (Brandle, 1999). However, many of them are still unknown.

Polyploidy

Polyploids are organisms that have more than one set of chromosomes above the diploid number of chromosomes. (Acquaah 2007; Chen 2010; Comai 2005; Ramsey and Schemske 1998). Polyploidy, which is found spontaneously in nature, has become a technique used by breeders by artificial induction. There are three reported advantages of being polyploid. The first two are heterosis and gene redundancy. These are results of gene duplication. The third advantage of polyploidy is asexual reproduction. While heterosis provides more vigorous plants compare to diploid plants, gene redundancy causes protection of polyploid plants from the harmful effects of mutations. Also, asexual reproduction allows to produce without a sexual partner.

Polyploidy breeding is a widely used technique for agronomic yield in economically important plants. With polyploidy, individuals have increased organ and cell sizes and they can be better adapted

to environment. Tetraploid plants are potentially more favored than diploids, with greater leaf size, thickness, increased mass and yield. In addition, secondary metabolites increase qualitatively and quantitatively (amount and quality) with the production of polyploid plants in medicinally important plants such as stevia.

Polyploid cells can occur after an inaccurate cell cycle (endoreplication, cytokinesis defect, mitotic slippage) or after cell-cell fusion. Also, it can be induced by some chemicals artificially. Polyploid plant is obtained with the use of chemicals known as antimitotic agents. Colchicine is the most common, oldest chemical used in chromosome folding, first reported as an antimitotic in the 1930s. Colchicine's chromosome folding occurs by attaching to microtubules and preventing the formation of spindle fibers. Colchicine tends to bind more to mammalian tubulins than to plant tubulins. Therefore, colchicine is more mutagenic in mammals than in plants. Recently, less mutagenic herbicides that can be used as an alternative to colchicine have attracted attention (Trifluralin, Amiprofos-methyl, oryzalin et al.).

These herbicides can be effective even at lower concentrations than colchicine. They are also more economical and less toxic than colchicine (Hansen et al., 2000). In addition to all these, less mix-ploid and chimeric plants were observed in polyploid plants obtained with other herbicides compared to colchicine (Zlesak et al., 2005).

Polyploidy Researches in Stevia

There are some studies that were tried to obtain plants with greater leaf size, leaf thickness, yield and glycosides by obtaining polyploid plants in stevia. Colchicine was used as an antimutagenic agent in all of these studies. Studies differ by factors such as

applied material (e.g. seed, seedling), method of application (e.g. dipping, soaking, spraying), dose and duration.

For this purpose, Shaopan et al., in 1995, tried to obtain polyploid plants by dipping the stevia growth tips 6, 8 and 10 times into colchicine at the rate of 0.04%, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 for 24, 48 and 72 hours. At the end of the study, the highest mutation rate was found at a rate of 31.25% in 8 times dipping application at a dose of 0.1% colchicine. It was observed that the survival rate decreased when the application time increased. As a result of this study, tetraploid plants were obtained, and 1.5 times more stevioside was found in the tetraploids.

In 2012, Mahadi applied colchicine to axillary buds at a rate of 0.25%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5% for 3 days in order to obtain polyploidy in stevia. As a result of the application, a total of diploid, triploid, tetraploid and mixoploid plants were obtained. Tetraploid plant formation has been reported at 1.0% and 1.5% applications. In the study, the highest glycoside ratio was found in tetraploids obtained with 1.0% colchicine.

In 2013, Yadav et al., in their study, kept the stevia seeds in distilled water for 24 hours and they removed the hairs of seeds. Then, these stevia seeds were exposed colchicine at the doses of 0.01%, 0.05, 0.1, 0.2, 0.4, 0.6% with 1% DMSO liquid solution at 22°C for 6, 18 and 24 hours. As a result, more than 0.23% colchicine was found to be mortal. In total, 4 tetraploid and 3 mixoploid plants were obtained. In the application of 0.2% colchicine for 6 hours, the desired tetraploids were obtained, but a high rate of mixoploidy was observed. 2 tetraploids were obtained at the rate of 0.6% and although no mixoploid was observed, only 5 of the 200 seeds applied survived.

In 2015, Ghonema et al. used both seeds and buds to obtain polyploidy in stevia. Seeds were exposed to colchicine at concentrations of 0.01, 0.05, 0.1, 0.25, 0.5% for 18 hours. For buds, one-month-old shoot buds were application was fatal. After the seed application, the survival rate and the total amount of glycoside in 2-month-old plants were observed at the maximum rate of 0.05% in colchicine application, but tetraploid plant could not be obtained. As a result of the bud application, the survival rate of the 2-month-old plants was 36% in the 0.05% colchicine application and a tetraploid plant could be obtained. In 0.01% colchicine application, tetraploid plant could not be obtained and the survival rate was reported as 37%. However, in both of the successful results of bud application, a decreased amount of glycoside was observed compared to the control plants.

In 2018, Hong Zhang et al. treated 2-day-old stevia seedlings with 0.05% and 0.1% Colchicine + 2% dimethyl sulfoxide (DMSO) at dark and room temperature conditions for 24 and 48 hours. As a result of the study, 10% and 20% tetraploid were obtained, respectively, in the applications of 0.05% colchicine for 48 hours and 0.1% colchicine for 24 hours, and an increase in stevioside and rebaudioside A were observed in tetraploid plants compared to control plants.

In 2020 Talei et al., in their study, the stevia seeds were treated with four concentrations of colchicine (control, 0.05%, 0.1% and 0.2%) for 12, 24, 36 and 48 h. As a result, they reported that the highest rate of chromosome doubling was achieved at 0.2% colchicine for 24 h, which was associated with the highest plant survival rates without toxic effects, while the explants treated with 0.1% and 0.2%

treated by spraying colchicine at concentrations of 0.01%, 0.05, 0.1, 0.25, 0.5% for 18 hours. As a result of the study, it was stated that the application of 0.1% or more colchicine in both bud and seed colchicine for 72 h were found as lethal. Also, they found the highest rebaudioside-A contents was at 0.2% colchicine and 24 h exposure time.

CONCLUSIONS

Stevia has the potential to become a basic crop as a natural sweetener thanks to valuable sweetening and medicinal compounds. The development of new *stevia* cultivars with high yield, resistance to biotic and abiotic stresses, high glycoside content and high germination rate is vital for the future of *stevia*. *Stevia* is a perennial plant and it has cross-pollinated structure. These factors make *stevia* breeding difficult.

The main methods used in *stevia* breeding are selection (especially recurrent selection), mutation, synthetic breeding, repetitive selection, and polyploidy breeding.

Polyploidy could potentially improve currently existing *stevia* cultivars or contribute significantly to the development of new cultivars in combination with other methods of *stevia* breeding. It is common to find an increase in glycosides in most *stevia* polyploidy studies. The polyploidy could be considered as useful method for improvement in medicinal and ornamental plants. However, in all the polyploidy studies carried out in *stevia* so far, no satisfactory results have been obtained and new studies are needed for more sensitive and precise information. Another shortcoming of *stevia* polyploidy studies is that they are limited only to glycoside

content. Other important characters to consider in stevia polyploidy studies are high leaf yield, high leaf-to-stem ratio, high adaptability to various environmental conditions, and high germination rate of seeds.

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EVALUATION OF SOIL TILLAGE SYSTEMS ON THE SCALE OF TURKEY

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Abstract

The aim of this research article is to provide readers with the results of previous studies on reduce tillage systems, and to make suggestions to the readers about the effects of direct cultivation on the properties of the soil, its advantages and disadvantages, at the scale of Turkey. It is estimated that the world population will reach 9.8 billion by 2050 and it is of great importance to manage the existing arable land in a sustainable way to meet the food demand of this increasing population. According to FAO, 1/3 of the world's agricultural land has become unproductive due to mismanagement. Studies have shown that there is an average of 150 tons/ha of soil loss per year in the world. In addition, the effect of increasing climate change in recent years has made the increase in soil degradation more severe in the arid and semi-arid regions of the world. In order to reduce this violence and improve the health of the soil, reduce tillage (RT), especially zero tillage (ZT), which is an alternative to conventional tillage (CT), is rapidly becoming widespread. As a result, it has been concluded that the application area of RT is quite limited in Turkey. For this reason, more research should be done to remove the obstacles in front of RT, farmers should be included in pilot studies and awareness should be raised. In this way, zero tillage can be applied in larger areas.

Key words: conventional tillage, no tillage, conservation tillage, soil properties

INTRODUCTION

The world population is estimated to be 9.8 billion in 2050. In order to meet the food needs of the increasing world population, it this increasing poverty, it is of great importance to reintroduce the existing degraded lands to agriculture.

One of the most important causes of soil degradation is tillage practices. CT causes the physical, chemical and biological properties of the natural structure of the soil to change. One of the best examples of this is the major reason for the loss of most of the US land between 1931 and 1939, the over-cultivation of the soil with plows and leaving the soil surface bare. During these years, thousands of people had to migrate to other places. At that time, it was understood that the over-cultivation of the land was not

is necessary to protect the existing lands and increase their productivity.

In today's underdeveloped and developing countries, the number of people struggling with deficit is increasing. In order to prevent sustainable. Therefore, soil protection systems have started to be researched, soil protection institutions and associations have been established (Anonymous, 2022).

If the soil continues to be over-cultivated and consequently lose soil fertility, this could lead to a global food crisis. As a result, people may have to migrate again. Estimates predict that 250 million people will migrate to Europe due to food shortages. This may be the second migration of tribes in the history of the world. This reveals the importance of sustainable use of soil. Many of the developing world countries such as Turkey

have either lost their productivity or lost some of their lands for various reasons by over-processing some of their lumps. Many studies conducted in Turkey have revealed that tillage systems Sustainable soil management is defined in the New World Soil Convention as follows:“Soil management is sustainable if the supporting, productive, regulating and cultural services provided by the soil can be maintained or improved without materially harming the soil functions or biodiversity that make these services possible.” (FAO, 2019).The State of the World Soil Resources report has identified 10 key threats to the success of Sustainable Soil

Management (STM) (FAO, 2019). These are given in Table 1. Some of these threats are closely related to tillage systems. have a great effect on this loss. In addition, climate change has brought changes in precipitation amounts and precipitation regimes together with temperature increases. These increased temperatures are strikingly more severe in arid and semi-arid regions. Conservation tillage is one of the measures that can be effective in this regard. Tillage has many negative effects on soil health. One of the most important of these is soil erosion. In order to prevent soil erosion, the soil surface should not be left bare.

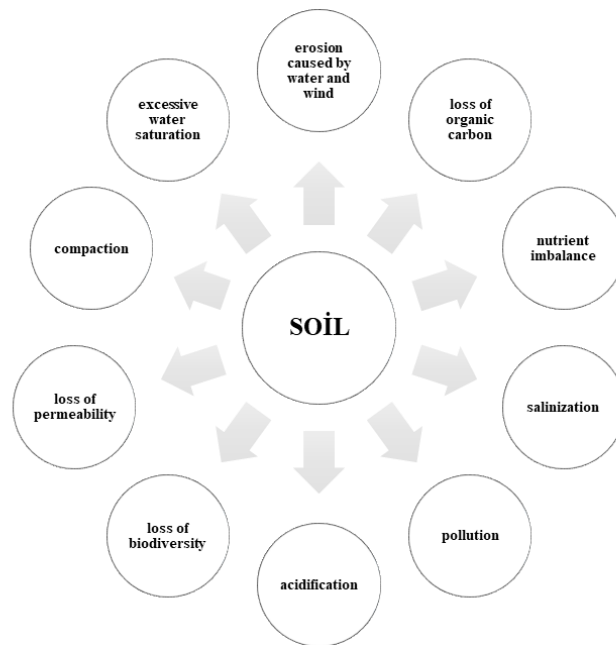


Figure 1. 10 threats to sustainable soil management

TILLAGE SYSTEMS

Different tillage systems have emerged based on the use of tillage tools with various effects. It is possible to collect these systems in two main groups; It is divided into two as conventional tillage and reduce tillage.

Conventional Tillage (CT)

In CT, if less than 15% of the soil surface remains and a plow is used, it is called conventional tillage. CT is first used by plows and the soil is cultivated at a depth of 25-30 cm

(Szostek, Szpunar-Krok, Pawlak, Stanek-Tarkowska, & Ilek, 2022). The soil is cut at this depth and turned upside down. Used agricultural tools; plow, disc harrow, crowbar, harrow, worshipful, seeder, sprayer, harvester

Goals and benefits of conventional tillage

- Optimizing the physical properties of the soil in terms of plant growth (relief)
- Mixing the organic residues (stubble, farm manure) from the previous production into

the soil (mixing, cutting, overturning, shredding)

- Destruction of weeds (cutting, knocking, shredding)
- Preparation of seed bed suitable for sowing (comminution, compaction)
- Control of erosion
- Preparation of the field for irrigation

Disadvantages of conventional tillage

- Organic materials in the soil decompose rapidly (Rahmati et al., 2020).
- Soil structure becomes monotonous from lumpy-granular state (Liu et al., 2022)
- More fuel is consumed (Kumar et al., 2021; Martins et al., 2021)
- CO₂ emissions increase due to burning stubble and aeration of the soil (Rutkowska et al., 2018; Shakoor et al., 2021)
- Since the field traffic is more, it causes soil congestion (Esposito et al., 2022; Takabatake, Fujisawa, Esteban, & Shibayama, 2020).

Organic matter content decreases at tillage depth (M. Li, Wang, Guo, Yang, & Fu, 2019), rapid water loss occurs at tillage depth.

- Soil cultivation has some disadvantages such as accelerating wind erosion and water erosion (Chappell et al., 2019; P. Li et al., 2018; Zhang et al., 2019).

Conservation Tillage

If the soil surface has more than 30% plant residues and is not cultivated with a plow, it is called reduce tillage. This system was developed to control soil erosion (Koller, 2003). Agricultural implements used in reduce tillage; It can be listed as disc harrow, crowbar, harrow, worshipful, seeder, sprayer, harvester.

Conservation tillage system is divided into main subgroups within itself.

These; It can be classified as strip tillage, plant tillage, mulch tillage, minimum tillage, zero (no) tillage.

Strip Tillage

It is a reduce tillage application that allows 1/3 of the field surface to be processed before planting for seed bed preparation. In this

application, tillage is usually done with planting. The soil is cultivated in strips 5 to 30 cm wide, the remaining areas are left covered with stubble (Godwin, 1990).

Plant Tillage

It is the system in which the stubble soil is cultivated and planted in one pass. It is used by combining the soil mill, rototiller and similar tools with the planting machine. In this application, if necessary, a chisel is attached to the front of the combination and the soil is worked by tearing it at a certain depth. Soil cultivation can be done in the whole field, or similar to strip tillage, only in the rows of planting.

Mulch Tillage

The basic philosophy of mulch tillage is to keep the soil surface covered with plant residues or vegetation all year round, to prevent the formation of a cream layer, to reduce shoot emergence problems and erosion. For this purpose, tools such as chisels, field cultivators, disc harrows are used. In addition to the performance of the sowing machine, the physical and chemical changes that occur in the seedbed region after planting affect the success of sowing the seed in the mulched seed bed (Aykas, Yalcın, & Cakır, 2005)

Minimum Tillage

Reduced tillage forms the subgroup of reduce tillage. In this system, chisel or disc tools are used for primary tillage, disc tools or cultivators are used for secondary tillage and seed bed preparation.

Benefits of conservation tillage

- It reduces the risk of erosion.
- It increases the infiltration of rain into the soil and reduces evaporation and keeps the moisture in the soil.
- It improves the soil structure by increasing the amount of organic matter in the top soil.
- It promotes biological life and activity in the soil.
- It saves time.

- It prevents high temperature and temperature change around the seed.
- It reduces fuel consumption by 40-50% as it limits mechanization operations to sowing in only one pass.
- It reduces greenhouse gas (CO₂) emission to the atmosphere.
 - It prevents the formation of cream layer, which makes the plant exit difficult and causes surface flow.
 - Since it reduces field traffic, it eliminates soil compaction due to this.

Disadvantages of reduce tillage

- Risk of product or yield loss
- Need for new equipment
- New disease and pest problems

- The field is not straight
- Difficulty mixing fertilizers and pesticides into the soil
- Increase in the use of agricultural pesticides with the increase in weeds
- Need for new information

Conservation tillage in the world and in Turkey

According to data from FAO, (2016), conservation tillage is increasing worldwide. There is no data for Turkey in 2004. When it comes to 2016, there is a significant increase in Turkey according to Table 2, however, this is a value of 0.03% relative to the existing cultivated agricultural land.

Table 2. Country-based area amounts of CT systems

Country	Area of Application of the CT System (ha) 2004/2005	Area of Application of the CT System (ha) 2016	% Increase
USA	25.304.000	35.613.000	41
Brazil	23.600.000	31.811.000	35
Argentina	18.269.000	29.181.000	60
Canada	12.522.000	18.313.000	46
Australia	9.000.000	17.695.000	97
Paraguay	1.700.000	3.000.000	76
India	-	1.500.000	-
Bolivia	550.000	706.000	28
South Africa	300.000	368.000	23
Spain	300.000	672.000	124
Venezuela	300.000	300.000	0
Uruguay	263.000	1.072.000	308
France	150.000	200.000	33
Chile	120.000	180.000	50
Colombia	102.000	127.000	25
Chinese	100.000	6.670.000	6570
Turkey	-	45.000	-
Others (Estimated)	1.000.000	143.253.000	14225

Studies on CT are increasing day by day. Especially in recent times when the effects of the global climate crisis have been experienced, the importance given to these studies has increased. In this graph, the studies carried out since 2015 (Figure 3) and the studies carried out from the past to the

present (Figure 2) are given on the reduce soil cultivation of the world countries. When we scan the web of science as 'conservative tillage' and 'soil', it shows the graph of the work done by the countries in this field. At the beginning of these countries; from United States, India, China.

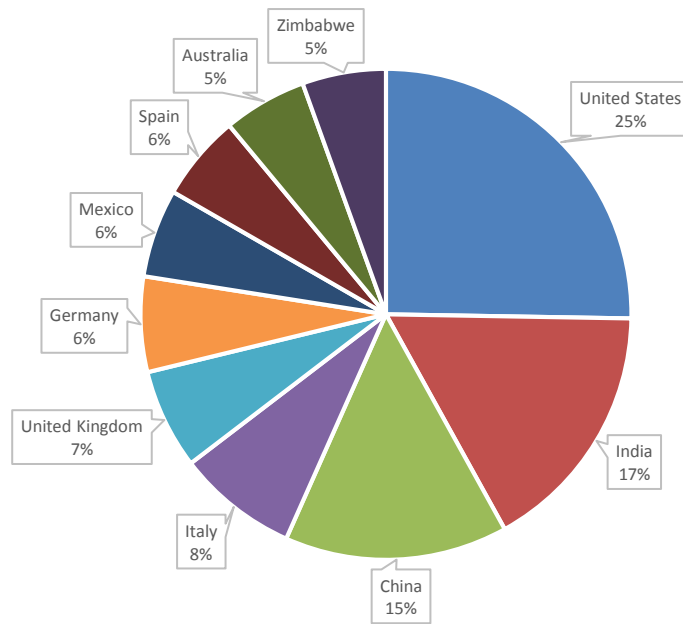


Figure 2. Top 10 countries doing the most research in the field of conservation tillage

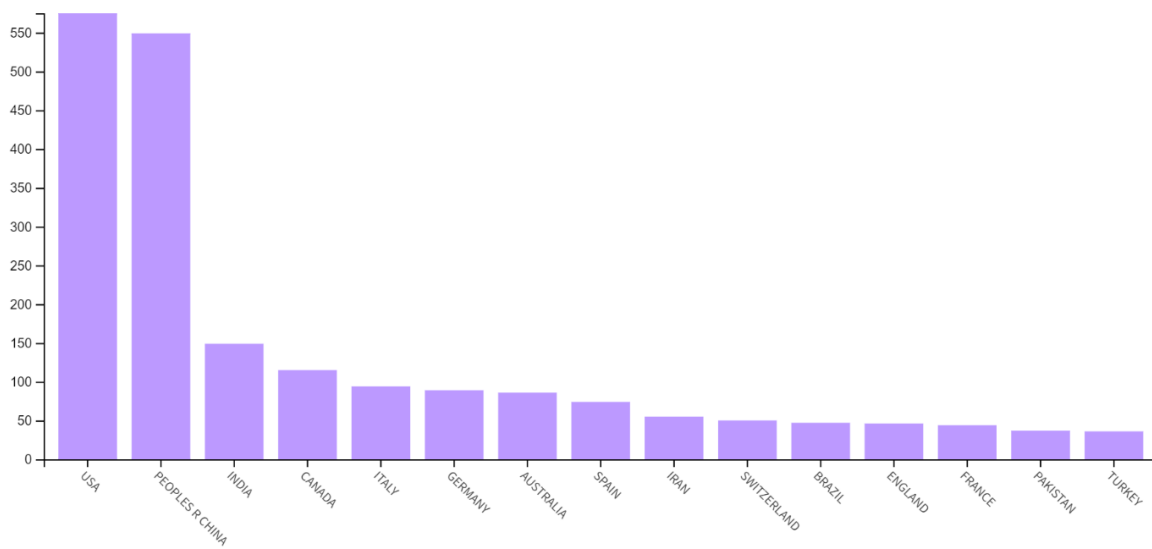


Figure 3. Number of studies carried out by countries on conservation tillage since 2015

As can be seen from table 2 and figure 2, 3 above, Turkey is far behind both in terms of work and practice. In addition, in parallel with the number of studies in the countries where the study was conducted, reduce tillage also increased.

Some studies carried out in Turkey are given below.

Günel and Çelik (2019) who have been working for 11 years by applying three different soil cultivation systems (CT, RT, ZT), in October 2017, after the maize harvest, 0-10, 10-20 and 20-30 cm depths

from each plot and undisturbed soil samples were taken and penetration resistance (PD) measurements were made in each plot. Aggregate stability, bulk density to evaluate soil physical quality, available water content and water-filled pore volume were determined. At all three depths lower aggregate stability were obtained in the traditional practice of burning stubble, and its values were increased together. The lowest bulk density were calculated for the reduced and zero tillage systems. In all tillage applications, PD values increased significantly with depth.

Yur and Yılmaz (2016), in a study they conducted, conventional, conservation and no-till sowing systems were tested in winter barley in 2010-2011 and 2012-2013 production seasons in Van province. Chisel plowing was observed to have a positive effect on soil penetration resistance, soil moisture content and root length density, and also on yield values.

Celik (2011), in his study, the effects of six different tillage systems on hydraulic conductivity, bulk density and penetration resistance in a clayey soil (Typic Haploxererts) in semi-arid Mediterranean climate conditions over a three-year period were evaluated. The research was carried out in three replications according to the random plot design. CT practices have increased hydraulic conductivity by reducing bulk density and penetration resistance in semi-arid climate conditions. The bulk density and penetration resistance values obtained in the zero-tillage application were found to be higher than the wheat producers in Konya did not know the zero tillage system at all and 57% were aware of it. In addition, in the villages where 300 kg/da of wheat was taken on average, 100 kg/da wheat was obtained due

cultivated soils and these values increased with the depth. Values indicative of soil compaction were found to be significantly higher at all depths under zero tillage and reduced tillage treatments compared to CT practices.

In another study was carried out during the growing season of winter wheat plant. Water consumption for winter wheat in no-irrigation conditions was 321.0 mm, while the total amount of net irrigation water required by the plant was 501.2 mm. When planting techniques were compared, it was discovered that the soil's 0-30 cm depth had the highest moisture content. The number of grain yield, straw yield, total yield, harvest index and grain moisture in terms of variance analysis among tillage techniques were not statistically significant. However results of direct drilling was higher than other parameters (Capar & Ucan, 2015).

Kucukongar, Kan, and Ozdemir (2014), in a survey study conducted in 2014, farmer behavior of zero tillage system and CT system in wheat in Konya province were determined. The study consists of the data obtained from the surveys conducted in March 2014 in the villages of Kulu, Cihanbeyli, Altinekin, Sarayönü and Çumra districts representing the dry and irrigated wheat cultivation areas in Konya. According to the results of the research, 2% of the total wheat planting area in Konya is planted with zero tillage system. and 13% stated that there was excessive weed in the field as a result of the zero tillage system. In the study, it was determined that 43% of the

to the wrong land selection, and this situation was perceived by the farmers in other villages as a huge decrease in yield with the zero tillage system.

Celik and Acar (2017), in their study, the effect of different tillage systems on total carbon content was investigated linked to aggregates at two different depths (0-15 and 15-30 cm) under long-term (2006-2014) wheat - corn and wheat-soybean rotation. A soil containing approximately 50% clay in Çukurova conditions. Each processing system is applied to an area of 480 m² and consists of 3 replication random plots. As the depth increased, the total carbon values in the aggregates under the reduce tillage (RT) systems decreased. RT systems have been found to provide significant improvements in total carbon bonding within aggregates compared to conventional tillage (CT) systems.

CONCLUSION

When we look at the amount of land where reduce tillage systems are applied in the world, the area applied in Turkey is very small, but intensive cultivation is carried out in areas where intensive agriculture is carried out. At the same time, it is not possible to compare the work of other countries. There are many reasons why the area where reduce tillage systems are applied is very small. The most important of them are; little work is done on this subject, the farmers are ignorant about it, the land structure in Turkey is divided into very small pieces by inheritance, and the technical tools are expensive. For this reason, preventive tillage studies should be increased and supported in state institutions, universities and private sector related to agriculture, land consolidation should be

Celik, I., (2011). Effects of tillage methods on penetration resistance, bulk density and saturated hydraulic conductivity in a clayey soil conditions. 17(2), 143-156.

Celik, I., & Acar, M., (2017). Çukurova koşullarında toprak işleme yöntemlerinin

made compulsory, and reduce tillage tools should be given more support. In case the Ministry of Food, Agriculture and Livestock allocates some of the agricultural support for input and product and analysis to those who implement the reduce tillage system within a certain pilot project, both the narrowing of the fallow lands and the expansion of the reduce tillage system will be possible. Although the results of the studies carried out in dry farming areas are successful in Turkey, there is a need for large-scale research and publication studies, including agricultural support policies, in order to expand the system in a sustainable way. In addition, a greater success can be achieved if the farmer who actually does this work is included in the work. In addition, universities and research institutions should lead the solution of problems by informing them through trainings. In addition, zero tillage can be much more economical when looking at increasing diesel and fertilizer costs. Because when the energy used in zero tillage is compared with the energy used in CT, a savings of over 60% is achieved. Thanks to RT, damage to water and environment can be reduced as well as soil conservation.

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DOUBLE HAPLOID MAIZE PRODUCTION

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Abstract

Maize is a plant widely used by humans with its rich content, as well as animal nutrition, and industry. Due to the ever-increasing food supply, it is a final goal to obtain the highest yield from the maize plant, like other plants, in the unit area. For this purpose, maize is a plant whose breeding has been done most intensively since the 1850s. Hybrid maize breeding is based on the establishment of homozygous lines, with traditional breeding methods, selfing is done during the season for 7-8 years. 100% homozygous lines cannot be obtained. Double haploid technology has been developed to shorten this process and to develop completely homozygous lines. In the double haploid technique, 100% homozygous lines are developed in 2 years. DH lines are used in the development of inbred lineages, genomic selection, mapping of quantitative trait loci (QTL), and generation of new genetic variations. Haploid plants are produced in-vitro and in-vivo techniques. However, adequate results cannot be obtained in in-vitro applications due to the genotype effect. Most of the commercially developed doubled haploid lines are which is obtained by the in-vivo haploid technique. The in-vitro technique, on the other hand, is less effective in developing doubled lines. In the in-vivo haploid technique, lines called inducers are used. Inducer lines are used as dust collectors in the production of paternal haploid plants and as pollinators in the production of maternal haploid plants. A primary donor is crossed with a plant-pollinator inducer line in maternal haploid production. Among the seeds formed, haploid plants are selected. The fastest and simplest method is the selection with the help of the R1-NJ color marker. Seeds with a colorless embryo and a purple endosperm are haploid seeds. The selected seeds are germinated and the chromosome number is doubled with the application of colchicine. 100% homozygous fertile lines are obtained. Haploid seed multiplication is achieved by selfing. However, the haploid rate is not high, it is a technique that needs to be developed.

Keyword: Haploid, double haploid, in-vivo

INTRODUCTION

Maize is a monocotyledonous plant belonging to the Poacea family. Maize is a diploid plant with $2n=20$ chromosomes.

Grains are grown in 721 million hectares of 1.5 billion hectares of agricultural area in the world. (Kardeş et al., 2019). Maize is one of the six cereals that is most produced in the world and meets the nutritional needs

of the world population with its versatile usage area, adaptability and productivity. (Anonymous 2015). Maize is largely used as animal feed in developed countries, while in less developed countries, more emphasis is placed on human nutrition. Worldwide maize production is consumed as 60% animal feed, 20% as human food, 10% as processed food and 10% as other consumption (Özata et al., 2013).

Maize is used as a raw material in the human nutrition starch, oil, glucose and feed industry with its rich nutritional content. Due to the rapid increase in the world population and its versatile usage areas, the demand for maize is increasing rapidly, and in parallel, it is necessary to breed high quality, high yielding, durable and adaptable varieties that will meet all these needs (Yorgancilar et al., 2019). For this reason, maize is the type of plant where breeding studies have been carried out most intensively since the 1800s. In Turkey, maize breeding studies were started in the 1950s. Many inbred lines and 42 maize varieties have been developed in Turkey with these breeding studies and 22 of these varieties are produced (Cengiz 2014). New varieties continue to be developed in Turkey and around the world.

Continuous development of new pure lines is required for the development of high yielding and quality hybrids in maize. The first and basic step in hybrid maize breeding is to obtain inbred homozygous lines. Pure line production is important in maize breeding as parents of hybrid lines. (Chaikam et al. 2019). In the variety development process, it takes the most time to obtain rootstocks. A period of 6-8 years is needed to obtain these pure lines with classical breeding methods. The homozygosity rate can reach 99% after 7 years of inbreeding. In order to shorten this period, other methods have emerged that complement and support classical plant breeding programs (Chase, 1969). Haploid production technique is a method used to obtain inbred lines. The disadvantage of this method is that the frequency of obtaining double haploids from haploids is low (Kermicle, 1969). Another disadvantage is the germplasm limitation, the low

probability of recombining the desired features of the original lines. However, the biggest advantage is the potential to obtain 99% inbred homozygous lines in a very short time. It is an effective technique for generating genetic variation within inbred lines. (Simmonds, 1983; Philips and Eberhart, 1993).

Haploid production is done by in-vivo and in-vitro methods. In maize breeding, double haploid technology based on in vivo haploid induction is recognized worldwide as an important way to increase reproductive efficiency. In recent years, the technology has been well applied in Europe (Schmidt, 2003), North America (Seitz, 2005) and more recently in China (Chen et al., 2009) with various commercial maize breeding programs. (Prasanna, 2012)

IN-VITRO AND IN-VIVO HAPLOID INDUCTION

Haploid plants can be produced by in-vitro and in-vivo methods. It involves obtaining haploids by tissue culture from megasporocytes and microsporocytes of maize in the in-vitro method. By culturing maize unpollinated ovaries in MS and N6 medium, approximately 2% haploid plants were obtained. (Ao et al. 1982). However, this method is rarely used because of its high dependence on parental genotype, high mutation probability during culture and long duration (Prasanna et al., 2012).

In-vivo method includes haploid induction with inducer lines. In-vivo maize haploid inducer lines are mostly derived from the same ancestral line, stock 6. Stock 6 originates from a Mexican ranch strain with white endosperm, a purple aleurone layer, and a hard core type (Meng et al., 2021). Because Stock 6 has a low induction rate in terms of agronomic properties, WS14

(Lashermes and Beckert, 1988), KEMS (Sarkar et al., 1994), MHI (Chalyk, 1999), RWS (Röber et al., 2005), Many inducer lines have been developed, such as and CAU5 (Chen, 2012).

Two different hypotheses have been proposed for the working mechanism of inducer lines: the single fertilization hypothesis and the chromosome elimination hypothesis (Meng et al., 2021). The first fertilization hypothesis was put forward (Sprague 1932). In this hypothesis, it was suggested that a sperm cell from the inducing gamete did not fuse with the egg cell but triggered haploid embryogenesis. According to the second hypothesis, the chromosome elimination hypothesis, two sperm cells from a single pollen of a haploid inducer line fuse separately with the egg cell and polar nuclei, but chromosomes from the inducer line are eliminated during subsequent zygotic divisions (Meng et al., 2021). Observations of micronuclei in ovules fertilized by inducer pollen and the presence of a DNA fragment from a rare inducer line detected in certain haploid seeds support this hypothesis (Li et al. 2009; Qiu et al. 2014). The two hypotheses do not contradict each other. The events in the two hypotheses can even occur simultaneously. Although research and evidence for chromosome elimination are more prominent, the single fertilization hypothesis remains valid.

Beckert (1994) compared in vitro and in vivo haploid plant production systems and stated that both methods are different from each other and have different advantages. He stated that it has advantages such as the formation of spontaneous dihaploids at a high rate in the invitro system, the high seed retention rate of the dihaploids obtained, and the fact that dihaploids are not followed

by a repetitive selection process. The use of the Inducer line is a method that enables the production of haploid plants in vivo to be simple, fast and cheap. The success of obtaining haploid plants with in vivo technique using inducer lines is between 2-15% according to the characteristics of the inducer line used (Röber et al., 2005). In the in vivo system, the absence of genotypic limitations makes it advantageous over the in-vitro system. However, the rate of spontaneous formation of dihaploids in vivo and the seed-binding abilities of the obtained dihaploid lines are low, and he emphasized that repeated selection for 2-3 years is required (Beckert, 1994).

Maternal haploid induction

In-vivo haploid breeding can be done maternally or paternally. In paternal haploid production, the inducer line is used as the main source line and the paternal powder donor line is used.

The in vivo maternal haploid breeding method consists of 4 steps: production of haploids, separation of haploids from diploids, artificial chromosome folding, and self-infection of folded haploids to obtain final lines.

Haploid Induction

Haploidy induction is carried out in the field or greenhouse. Plants of the haploid stimulator and source germplasm are grown under conditions conducive to plant growth, pollination and maturation of seeds. By adjusting the sowing dates of the inducer and the source germplasm to synchronize the pollen shedding with the shaking of the source germplasm, simultaneous development is achieved and successfully fertilized seeds are obtained. It can be closed with a protective paper bag for controlled fertilization of the cobs on the

plants of the source germplasm and not to get dust from the outside. For large-scale haploid induction trials, open pollination is provided by planting the source plants according to the trial design with inducer lines. However, all source germplasm plants should be tasseled to avoid pollen contamination during pollination with the inducer. (Prigge and Melchinger, 2012).

Haploid Definition

It is necessary to select haploid seeds or plants from the cobs obtained after crossing the starting materials with the reducing lines. Since haploids are present in a very low proportion compared to diploids, it is very important to separate haploids from diploids before artificial chromosome doubling. Seeds with 3 different phenotypes are formed. Making this distinction during the seed period reduces labor and reduces costs. There are many haploid identification methods such as identification of haploid plants, measurement of pollen lengths with a flowcytometry device, counting of chloroplasts in epidermis cells, determination of chromosomes by karyotypic studies, and use of R1-nj color marker. The R1 -nj marker is the most widely used and easiest color marker for haploid identification at the seed stage. (Meng et al., 2021) Anthocyanin marker gene R1-nj gene is used as a marker in inducer lines. The dominant gene, R1-nj, provides purple coloration in the aleuron part of the seed. Anthocyanin color allows to phenotypically distinguish haploid seeds from contaminated seeds and diploid seeds (Yorgancılar et al., 2019). Seeds with normal embryo and endosperm color are contaminated seeds, that is, non-haploid diploid seeds that have foreign dust. It has a very small proportion in the selected

population. Seeds with purple embryos and purple endosperm are diploid seeds formed as a result of normal fertilization with the reducing line. Seeds in this category have the highest total percentage of seeds. Finally, seeds with colorless embryos and purple endosperm are seeds that represent haploid embryos (Cerit et al., 2016). It is used to pass the folding stage with seeds.

Artificial Chromosome Doubling

Haploid plants are sterile, sterile plants. Since they have a single set of chromosomes, meiotic divisions and gamete formation do not occur in haploid plants. For meiosis to occur, homologous chromosomes must be separated from each other. However, homologous chromosome pairs cannot be found in haploid plants. In order to reproduce haploid plants, the number of chromosomes must be doubled. During mitosis, sister chromatids are prevented from separating from each other. By preventing cell division, chromosomes that match themselves in mitosis do not separate from each other and full homozygous alleles are obtained. Colchicine and other chemicals with anti-mitotic activity that prevent microtubule formation are used in chromosome folding (Chaikam and Mahuku 2012).

Haploid seeds are germinated on paper towels until the coleoptiles reach 2 cm in length. The coleoptile tip is cut before being dipped in colchicine-containing solution to facilitate absorption of the doubling chemicals. Next, the seedlings are washed under water, planted in a greenhouse or biodegradable pots for transplanting up to the three-leaf stage, and then transplanted into a DH nursery site (Prasana 2012; Prigge and Melchinger 2012).

Selfing for Production of Double Haploid

Finally, in obtaining pure lines, putative double haploid plants are planted to protect and propagate seeds of new DH lines. It is done by selfing. However, the application of colchicine causes stress in the seedlings. The formation of healthy seeds from plants under stress is not expected. For this reason, in the production of double haploid seeds, plants should be grown by protecting them from stress factors and very careful attention should be paid to prevent genotype losses. Also, colchicine treatment does not result in uniform or complete doubling of chromosomes in all cells of a seedling. The effect can be variable, especially on the tassel: some plants may have copious pollen-producing tassels, while in most cases there is no or limited pollen production on the tassels. Self pollination can be extremely difficult. Plants must be protected from diseases, pests or other destructive environmental factors (Prigge and Melchinger 2012).

CONCLUSION

Although the use of haploid techniques in maize breeding provides many advantages over classical plant breeding, it still does not have the desired level of haploid induction rate. At the same time, double haploid seedlings are not highly adaptable to field conditions. With the development of new inducer lines and the diversification of the use of techniques, these problems may disappear over time. Breeding periods for maize, which is an important plant, can be reduced from 10 years to 2-3 years. New research on haploid induction rates and inducer lines will further expand the use of in-vivo double haploid methods in maize breeding.

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THE EFFECT OF PRIMING PROCESS ON ROOT AND SEEDLING DEVELOPMENT OF SOME FOOD LEGUMES SEEDS

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Abstract

Edible legumes, which are from the "Leguminosae" family, are one of the plant-based foods that are important in human nutrition. Edible legumes; have superior features such as containing high levels of absolutely essential amino acids (lysine), not containing cholesterol, having low fat ratios, being rich in microelements and vitamins. Various applications to the seed before sowing in order to minimize the problems that may occur during germination and emergence due to adverse environmental factors or directly to the quality and structure of the seed, to ensure uniform seedling emergence and strong seedling development in a short time, and in order to increase resistance to stress conditions, various applications made to the seed before planting are called "priming" in general. In this research, Native Red Lentil seeds belonging to the "Lens culinaris" species were used as plant material. For application priming %1 KNO_3 , %2 KNO_3 , %3 KNO_3 doses were used. And only pure water was used as control. Seeds were kept in KNO_3 doses and pure water for 18 hours at room temperature. At the end of 18 hours, the seeds were planted in viols. Among priming application; root number, root length, root weight, total leaf weight, stem length, seedling length and seedling weight parameters were measured. Root weight, total leaf weight, stem length, total number of leaves and seedling weight were examined statistically significant examined. We recommend our farmers to use %3 KNO_3 priming dose in their lentil production.

Key Words: Priming, edible legumes, lentil, KNO_3

INTRODUCTION

Edible legumes, which are from the Leguminosae family, are one of the plant-based foods that are important in human nutrition. Edible grain legumes: They have superior properties such as containing a high percentage of absolute necessary amino acids (lysine), not containing cholesterol, being low in fat, being rich in microelements and vitamins. Edible grain legumes enrich the soil in terms of nitrogen by binding the free nitrogen of the air with Rhizobium bacteria that form nodosity in their roots. After harvesting the plant, a high

amount of nitrogenous organic compounds contained in the roots are broken down by microorganisms in the soil, some of which decomposes, and then the planted plants benefit from this nitrogen. In addition, legume roots aerate the soil, prevent soil compaction, improve the physical, chemical and biological properties of the soil and contribute to the maintenance of soil fertility. Among the edible grains of legumes in Turkey, chickpeas take the first place in terms of cultivation area and production, followed by lentils, beans and

Pods respectively. Turkey, especially chickpeas and lentils in the world is among the most important producing and exporting countries. In order to minimize the problems that may be experienced during germination and exit depending on the negative environmental factors or directly the seed quality and structure, to provide uniform seedling output and strong seedling development in a short time and to increase resistance to stress conditions, various applications made to seed before sowing are generally called "Priming". Obtaining the desired plant frequency and therefore high yield depends primarily on the germination and output of the sown seed in a short time and at a high rate. Depending on both environmental and genetic factors and seed structure, seeds are subjected to various applications called priming in general before sowing in order to eliminate the negativities that may be experienced during germination and exit, to obtain an adequate seedling plant and yield. In many plant species, especially in inappropriate conditions such as low temperature, priming increases the germination-exit rate and speed, allowing seedling to be established as often as desired in a short time. As a result of priming application, rapid root and shoot exiting allows for stronger seedling development and yield increases. New cultivation systems are being worked on that will eliminate or

minimize the yield and quality losses that occur in the cultivation for environmentally friendly production techniques. In this regard, priming methods are applied in order to improve the quality and performance of seedlings in solutions prepared using organic preparations.

MATERIALS AND METHODS

In my study, seeds of Native Red Lentils belonging to *Lens culinaris* were used as production material. 1% KNO₃, 2% KNO₃, and doses of 3% KNO₃ were used. And only pure water was used as control. The seeds were soaked in KNO₃ doses and pure water at room temperature for 18 hours. At the end of 18 hours, the sowing of seeds was carried out on violas. Our trial was carried out in the laboratory at room temperature. The number of roots, and root weight were measured as belonging to root development. The total leaf weight, and the length of the seedlings were measured as pertaining to the development of the seedling. In addition, for measurements; We used precision balances, rulers, volume meters.

RESULTS AND DISCUSSION

Root weight and total leaf weight, and were examined statistically significant examined. We recommend our farmers to use 3% KNO₃ priming dose in their lentil production.

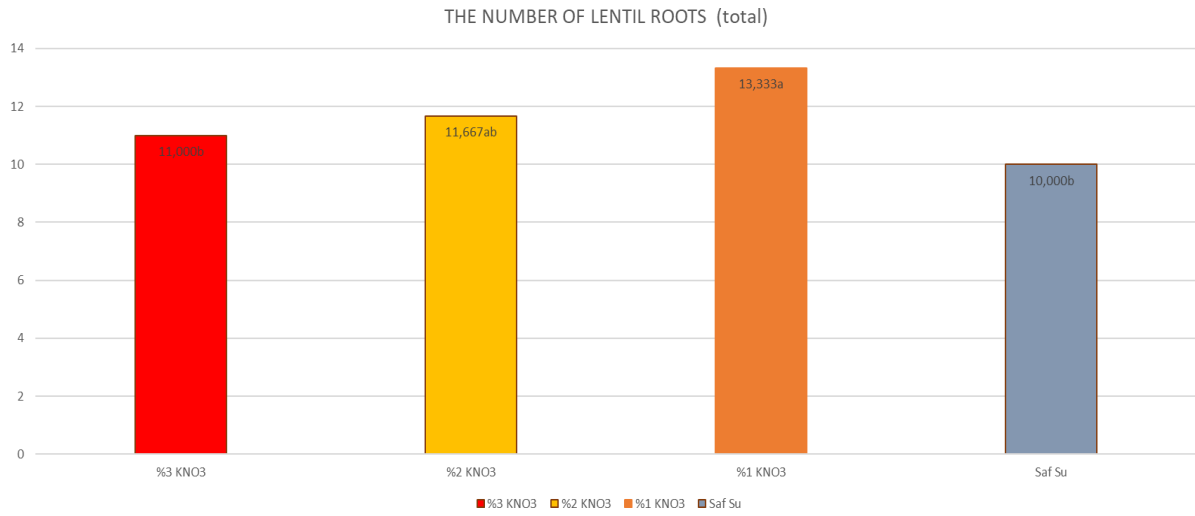


Figure 1

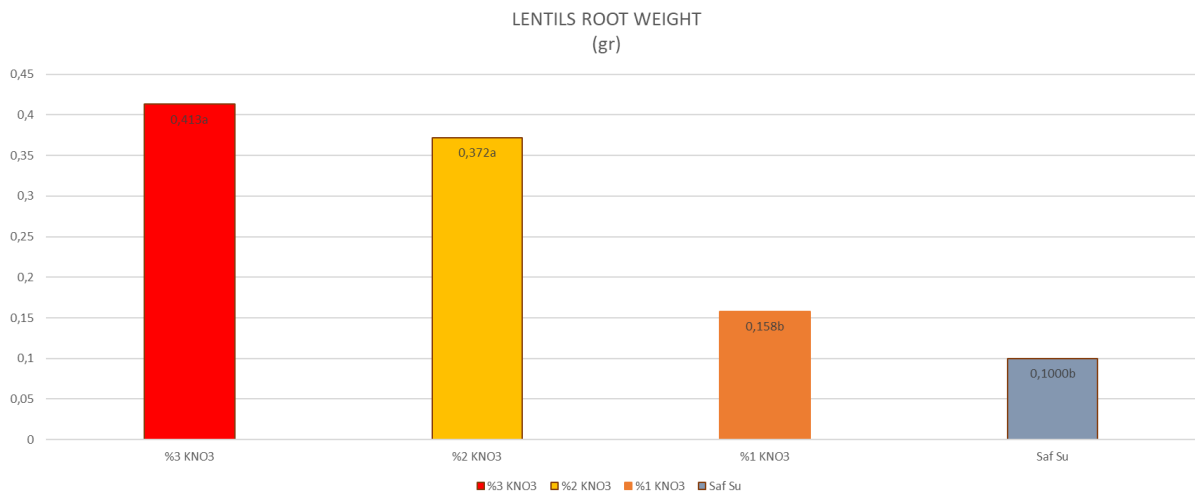


Figure 2.

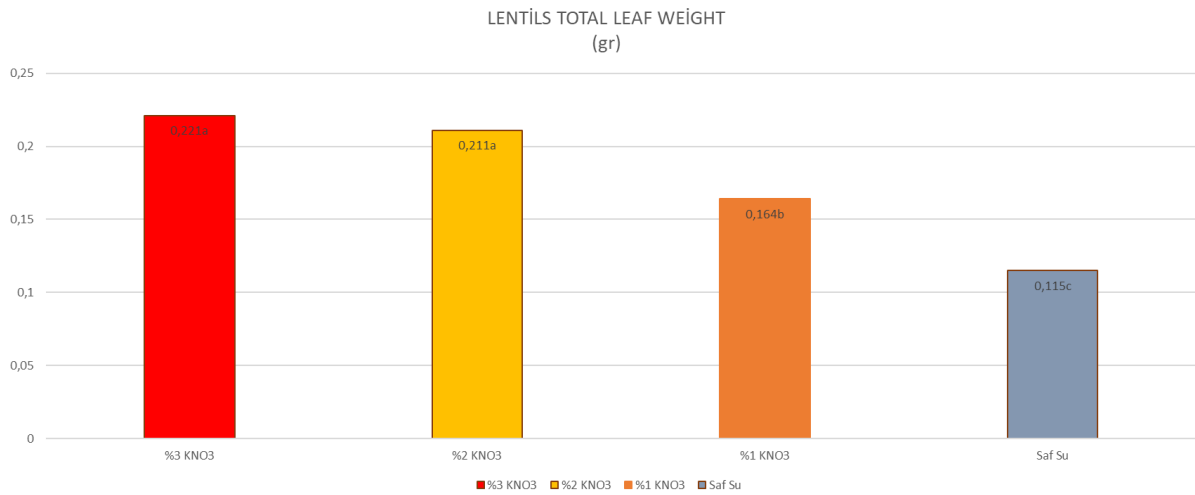


Figure 3.

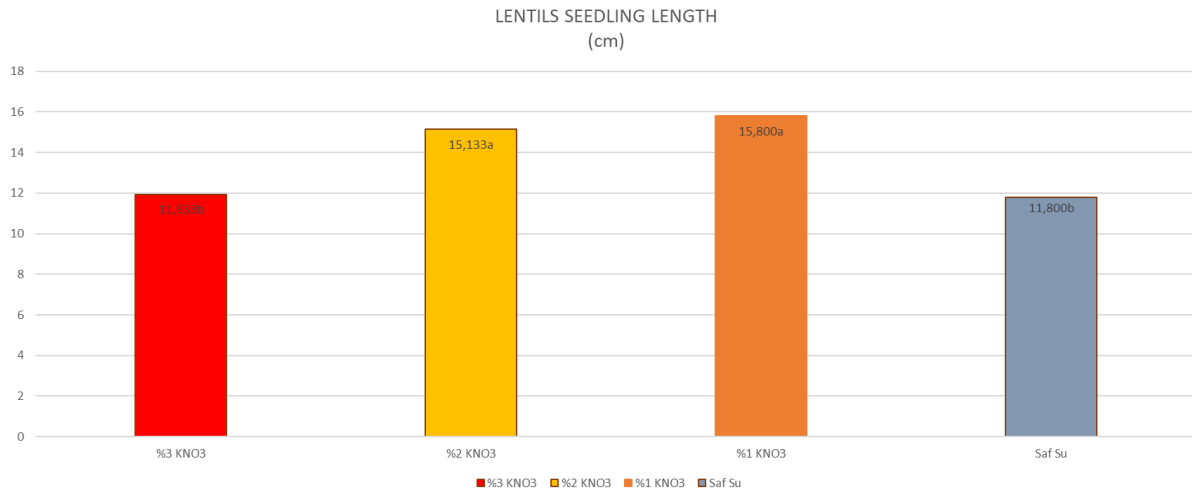


Figure 4.

Ercan ÖZKAYNAK et al. (2020) in their study plant height, root length and plant age weight values both in the seeds of tomatoes and peppers, and in different priming applications (thyme, moss, laurel, control) different. What the applications have in common is in priming applications to control plants earlier than germination in the water used, more faster, more homogeneous and stronger seedling development provided. In a similar study, the seeds collected 70 days after the annexation showed that the embryo had water creating sufficient osmotic potential to increase the coverage of the primary root more it has been shown to come out well from previous harvests (Hegarty, 1978). on germination could be due to osmotic effect rather than specific ion accumulation. Mehra et al. (2003), PEG molecules to seed and therefore the seed will not continue to be absorbed when the water potential of the seed and its surroundings comes into equilibrium. Özmen, K. and Kenanoğlu, B.B (2020) in their research, the change in the length of the root and shoot of the Aydin black variety in the control group. When it is looked at, it has risen although it is fluctuating depending on the maturity

With these results, cabbage and rapeseed Still and Bradford in seeds (1998), Welbaum and Bradford in melon (1990), eggplant Iron and others in seeds (2003) also found that they are more sensitive to osmotic potential and salt stresses exerted on unmaturing seeds than mature period seeds they are determined. Both osmotic and toxic effects of salts affect germination performance (Allen et al., 1986). Iron and its companions (2008), peppers of different ripeness in the work they do with their seeds; When the same harvest period and its results at concentrations were compared with PEG, the germination percentages were higher with NaCl application and it was revealed that the negative effect of PEG periods. In general, a decrease in the length of roots and shoots at all ripenancies was detected, except for the thermoprimering group, which was performed for 15 minutes at 50 °C. Seeds harvested on the 50th day after flowering (raw seed) due to the inability to complete physiological maturity, the consequences are quite variable and less in length. Root and shoot lengths belonging to the 55th and 60th day harvest groups in the Kemer 27 variety an increase was determined according to the

results of the control group with thermoprimer applications. Kazemi Afshar & Çelen's research (2021) When looking at the seed yield in sunflower, plant, there are significant differences between applications at the statistical level and the highest seed yields per plant were obtained from priming KNO₃+GA₃ + Coating and priming KNO₃ applications with 113.7 and 112.3 g respectively. The lowest seed yield per plant is 95.7 and 95.3 g, respectively, it has given control and coating applications. Toklu (2017), in her research KNO₃ priming applications to cotton seeds had an increasing effect on characteristics such as plant height, root dry weight and total dry matter amount in potting conditions, and according to the results of the research, all priming applications caused a significant increase in seedling growth rate compared to the control application. SG 125 and Flash cotton varieties to seeds research results in which they examined the effects of some priming practices, mannitol, germination of pure water and applications such as KNO₃ and that it provides advantages over the control application in terms of seedling development shown.

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THE EFFECTS OF DIFFERENT LIGHT INTENSITIES AND COMPOSITIONS ON YIELD, QUALITY AND OTHER PARAMETERS OF BARLEY FODDER IN VERTICAL FARMING SYSTEMS

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Abstract

According to the Food and Agriculture Organization of the United Nations (FAO) report, the most pressing problem is the concern of not being able to meet the increasing demand for a safe, adequate and suitable food supply for the growing population. The same report underlines that by maintaining existing farming practices, there will not be enough space to meet the world's growing food needs. For this reason, problems such as climate change and drought and extreme weather events that rapidly increase their effects reveal that agricultural resources should be used more effectively.

Vertical agriculture is an alternative method to the efficient use of resources (for example, 70-80% less water) in order to use the unit area much more efficiently than traditional agriculture. Since the transportation problem is eliminated in vertical farms established in the city, exhaust emissions to nature are also reduced. Weather events that affect crop yield in traditional agriculture and are not under the control of the producer have been eliminated in vertical farming systems. Based on the type of plant to be grown in the system, production throughout the year can be programmed on the basis of demand and plant growing conditions can be optimized. Efficiency can be maximized by fine-tuning the temperature, humidity and lighting conditions.

This review article draws attention to the fact that all the reasons I mentioned above, lead us to find more innovative and environmentally friendly agricultural techniques and that the studies in this field are gaining momentum.

Key Words: vertical farming, sustainability, barley fodde

INTRODUCTION

Insufficient, balanced and efficient feeding of our animal stock in Turkey is among the main problems of the livestock and agriculture sector. One of the reasons for this problem is the shortage of quality roughage. If we add the diminishing resources due to the impact of climate change and the destructive aspects of traditional agricultural models, it is understood that we need to approach this problem with a more environmentally friendly, innovative and technological method.

Vertical agriculture, which is a new and alternative production method, has been developed in order to be a solution to these problems and other possible problems that may arise in the near future. In the current thesis based on this innovative idea; In this study, it was aimed to grow the forage barley grass selected to close the roughage deficit in the vertical agriculture growth module to be created and to determine the required optimum light intensity and composition, and then to reveal its economic analysis. The objectives determined in line with these objectives are listed below:

a. Establishment of the prototype conditions of the vertical farming system: In the prototype of the vertical farming system, necessary to ensure the highest growth and feed quality: it is aimed to determine the optimum water application and optimum temperature conditions

b. Determination of barley varieties suitable for vertical farming system: It is aimed to determine the most suitable barley varieties to be grown in vertical farming system in terms of resource usage need, growing period and nutritional values.

c. Evaluation of the prototype in terms of resource use and energy efficiency: Calculation of direct and indirect energy use of the prototype; It is aimed to calculate the efficiency of use of resources such as water, electricity, seeds and the environmental consequences of the inputs of the system, such as carbon and water footprints.

d. Demonstrating the economic analysis of the prototype: Starting from the prototype; It is aimed to determine the gross product, operating costs, real expenses, net product, agricultural income values of 1 decare barley grass vertical farming system.

In the light of the results to be obtained with the current thesis, it is aimed that the vertical agriculture prototype to be created will contribute to the production of high quality roughage in Turkey, and develop a sustainable technology that produces uninterruptedly all year round, uses environmental resources at a minimum level.

The fact that the world population will reach 9.8 billion in 2050 (United Nations, 2017), limited natural resources and environmental problems such as global climate change bring the issue of global food security to the agenda intensively. According to the Food and Agriculture Organization of the United

Nations (FAO) report, the most urgent problem faced is the necessary for the increasing population; It is revealed that there is a safe, sufficient and affordable food supply (Besthorn, 2013). In the same report, it is underlined that there will not be enough space to meet the world's increasing food needs by maintaining existing agricultural practices and even with the best efforts in sustainable land use. On the other hand, the rapidly increasing climate change and the problems it creates such as drought and extreme weather events reveal that agricultural resources should be used more effectively (Jia, 2019). In this context, developing technology and technological equipment that is getting cheaper day by day; In the field of agriculture, research and applications for the efficient use of resources and their evaluation for environmentally friendly and sustainable food production are increasing rapidly in the world (Jia, 2019).

In addition to the widespread use of technological applications integrated with existing agricultural systems such as agricultural machinery control systems, equipment automation, remote sensing methods, use of drones, agricultural information system, farmer portal, electronic tagging, it offers a different agricultural production system such as vertical farming. technology applications are also planting their first seeds in the developing world.

The vertical farming system is based on the principle of growing plants by vertical stacking with low agricultural input, using technological elements, and is presented as an important potential by different disciplines, from agricultural experts to urban and regional planners (Despommier, 2009). (Besthorn, 2013). Currently,

commercialized vertical farming practices; It is found in countries such as America, Germany, South Korea, Japan, China, Singapore, Italy, Netherlands, England, Jordan, Saudi Arabia, United Arab Emirates and Canada (Besthorn, 2013). Accordingly, the vertical agriculture market, which exceeded 4.5 billion dollars in 2020, is expected to increase by 23% until 2027 and reach a value of 19 billion dollars (Pulidindi and Prakash, 2020). In our country, the number of researches and applications carried out in this field is almost negligible. Today, plant groups that are widely grown with the vertical farming system are mostly; lettuce, cabbage, spinach, basil, arugula etc. They are plants whose leaves are used (Şahin and Kendirli, 2016). However, many studies and applications are rapidly spreading about the production of other herbal products with such systems. When our country is evaluated in terms of our agriculture, it is seen that the use of vertical farming system for this purpose has an important potential when it is considered that the most important problem is the feed problem and our roughage deficit has reached 30 million tons (Özkan and Demirbağ, 2016). The increase in livestock, the inability to find products of the same quality throughout the year, input costs such as fertilizers and pesticides, inadequacy of water resources and natural events caused by climate change are among the biggest obstacles to farmers' production (Kumar et al., 2018). In this framework, in the current thesis, growing barley grass for roughage production by using vertical farming technology is seen as an important potential for solving the problem both in terms of efficient use of environmental resources and ensuring sustainability. From this point of view, when the studies on growing barley

grass indoors are examined, quite different suggestions have been encountered in terms of the environmental conditions created. For example, the amount of water supplied to the system; It varies greatly from 7,000 ml/m².day (Al-Karaki and Al-Hashimi, 2012) to 91 ml/m².day (Ajmi et al., 2009). When evaluated in terms of water use, it is stated that up to 80-90% water savings can be achieved in the vertical farming system (Despommier, 2013), therefore, determining the optimum water use and values in the envisaged barley grass growing system is of great importance, especially today, when the problem of water scarcity is on the agenda. There is almost no study (Karaşahin, 2017) on the determination of the barley variety used as a seed in the system. Determining the most suitable variety in terms of feed quality is also important in terms of outputs such as animal health and milk yield.

In the light of the researches examined, 20-30 kg/m² of fresh barley grass is obtained for animal feed in approximately 7 days (Al-Karaki and Al-Hashimi, 2012; Castillo et al., 2013; Dung et al., 2010; Fazaeli et al., 2011). Based on these data, it is predicted that the annual fresh barleygrass production will be around 5,180-7,700 tons in a one-decare vertical farming facility, which is the subject of the prototype designed for the thesis work and can be reached in the future, with uninterrupted production throughout the year. When this value is compared with the average 5.2 tons/da silage corn yield according to TUIK (2021), it corresponds to 1.057-1,571 da of silage corn cultivation area. However, the efficiency and economic level of the designed system should be determined by the clear determination of barley grass growing conditions; It is directly dependent

on the optimization of inputs such as seed, water and energy requirements. For this reason, in addition to the development of the prototype, optimization and economic analysis of resource use is very important in terms of the benefits of the study.

Along with this, today's concerns about reducing greenhouse gas emissions in order to reduce the negative impact of climate change and ensure food security have recently increased the interest in assessing impacts such as carbon and water footprints for various activities and products (Hertwich and Peters, 2009). In this context, the calculation of the carbon and water footprint of the prototype to be developed is important for the evaluation of environmental sustainability.

With the current thesis, a sustainable environment-oriented system is envisaged in which water savings of up to 80-90%, fertilizer and pesticide costs are reduced to almost non-existent levels, soil tillage and diesel use are eliminated, compared to traditional methods, roughage production. In our country, scientific studies on vertical agriculture are very limited and studies for feed purposes are very few. It is expected that this study will have a significant widespread impact on a regional and national scale by presenting a low-input and environmentally friendly production system, especially to regions that are not suitable for agriculture or to large and medium-sized livestock enterprises. Considering all these evaluations and possible results, the importance of the study is revealed.

MATERIALS AND METHODS

The researches will be carried out under fully controlled conditions in the cassette type container to be created for the design

of the vertical farming prototype. The prototype will be made of 50 mm carbon reinforced insulated sandwich panel, 110 x 500 cm (5.5 m²), 255 cm high. In line with the 4 main objectives planned in the project, the physical conditions of a growing tray will be as listed below:

a. Growing tray dimensions: 50x100x7 cm (0.5 m²). There will be a total of 45-50 germination trays in the prototype.

b. Growing tray specifications: PP, PE, ABS, PC Plastic

c. Lighting: Led lighting will be used in the light intensity and composition determined within the framework of the preliminary studies to be carried out (currently 2 master studies are carried out on this subject), which will pass over the growing trays and will be placed on the line (height = 50 cm). The illumination duration will be controlled by a timer with 18 hours of light and 6 hours of darkness (Pedretti, 2013).

d. Irrigation-humidification: A spray irrigation system will be used to pass over the growing trays and be placed on the line.

e. Temperature-humidity monitoring: It will be recorded instantly with 2 recording devices to be placed in the prototype and monitored on the display.

f. Temperature control: Air conditioning unit will be used.

g. Renewable energy source (solar panels) will be used to support sustainability for lighting, irrigation and air conditioning.

RESULTS AND DISCUSSIONS

1. Trial factors *i.* different water applications and *ii.* determined for different temperature conditions. Attempt; In Random Plots, it will be carried out in 3 replications according to the Divided Plots Trial Pattern. Considering the interactions between factors, a total of 900 growing trays; measurement, analysis and evaluation

will be taken. A total of 20, 8-day cultivation periods will be carried out.

Water applications will be carried out at 400, 500, 600, 700 and 800 ml/m².days within the framework of the results obtained by Al-Karaki and Al-Hasimi (2012) and Ajmi (2009). Irrigation will be carried out as a spray over the growing trays (height = 50 cm).

Process: Each trial will take a total of 8 days. Different varieties of barley seeds will be distributed homogeneously on the growing trays in the amount to be determined in the preliminary studies. From the first day, the relative humidity of the air in the prototype will be kept at 70-80%, and spray irrigation will be carried out from the tray top cover so that the seeds are constantly moist but not under water. The amount of water supplied to each growing tray will be recorded. The following measurements and analyzes will be performed, respectively, using samples taken from the trays every day:

Wet weight (g/m²): Plant samples will be taken from a 5x5= 25cm² area of each growing tray, the number of plants will be counted and the wet weight per square meter will be calculated by weighing the wet weights.

Pigment analysis (mg/g): 1 gr. to be taken randomly from the sample. In the wet sample, chlorophyll a, chlorophyll b and carotenoid analyzes will be performed according to Arnon (1949) and Jayaraman (1988) using a Cary 50 brand UV/VIS spectrophotometer.

Leaf Area: The leaf areas of 10 plants to be taken randomly from the sample will be calculated based on the separation of RGB color values using digital photography and Adobe Photoshop software.

Dry weight (g/m²): The remaining samples will be kept in an oven at 105 °C for 24 hours, with their roots and stems separate, and their dry weight will be determined and the dry weight value per square meter will be calculated.

Proportional Growth Amount (RGR mg/g.day): RGR (Relative Growth Rate) value will be calculated using the formula below, based on the dry weight values obtained every day (Blackman, 1919).

$$RGR = (\ln W_2 - \ln W_1) / (T_2 - T_1)$$

W1 = initial dry weight

W2=final dry weight

T1= start time

T2= end time

Product Growth Amount (CGR g/m².day): Based on the dry weight values obtained every day, the CGR (Crop Growth Rate) value will be calculated according to the formula below (Watson, 1952).

$$CGR = ((W_2 - W_1) / (T_2 - T_1)) \times (1/P)$$

W1 = initial dry weight

W2=final dry weight

T1= start time

T2= end time

P = unit area

Net Assimilation Rate (NAR g/m².day): Based on the dry weight and leaf area values obtained every day, the NAR (Net Assimilation Rate) value will be calculated according to the formula below (Gregory, 1918).

$$NAR = (W_2 - W_1) / (T_2 - T_1) \times [(\ln LAI_2 - \ln LAI_1) / (LAI_2 - LAI_1)]$$

W1 = initial dry weight

W2=final dry weight

T1= start time

T2= end time

LAI1=initial leaf area index

LAI2=final leaf area index

Leaf Area Ratio (LAR cm²/g): Based on the dry weight and leaf area values obtained

every day, the LAR (Leaf Area Ratio) value will be calculated according to the formula below.

$$\text{LAR} = \text{W}/\text{LA}$$

W = dry weight

LA = leaf area

Water Use Efficiency Based on Irrigation Water (WUE_{IR} mg/g): Irrigation water use efficiency (WUE_{IR}) will be calculated based on the following formula (Roth et. al., 2013):

$$\text{WUE}_{\text{IR}} = \text{W}/\text{IR}$$

W= total dry weight

IR = total amount of water supplied

Nitrogen Content (mg/g): In the samples to be obtained after the last harvest, the amount of crude protein will be calculated based on the Nx6.25 calculation from the amount of nitrogen (N) to be obtained by the Kjeldal method (Helrich, 1990).

Feed quality analysis: From the dry samples of the last harvest, 1 g x 4 samples will be kept in the oven at 550 C for 24 hours to determine the amount of ash. Again in the last examples, Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF) and Acid Detergent Lignin (ADL) analyzes will be performed with Fiber Analyzer (ANKOM) device (Van Soaest et al., 1991).

2. Trial factor is temperature applications, and considering the studies of Fazaeli et al., (2012) and Akbağ et al., (2014), it will be applied at 4 levels with an average of 17±1, 20±1, 23±1 and 27±1.

Process: Temperature control will be provided by a digitally controlled air conditioning unit. As in the other experiments, barley seeds of different varieties will be distributed homogeneously in the amount to be determined in the preliminary studies. It will be ensured that the relative humidity in the prototype is kept at the level of 70-80% and the seeds will be

sprayed over the growing trays (height = 50 cm) in such a way that they are constantly moist, but not under water. The amount of water given to each tray will be recorded. The following measurements and analyzes will be performed, respectively, using samples from the growing trays each day:

The details of which are given in the water application (1st Trial) section; number of plants and fresh weight (g/m²), dry weight (g/m²) and leaf area (cm²) measurements, as well as RGR (mg/g.day), CGR (g/m².day), NAR (g/m². days) and LAR (cm²/g) calculations will be performed. Crude protein (mg/g), ADF, NDF and ADL analyzes will be performed in the post-harvest samples (Detailed information about measurements and analyzes is given under the title of 1st Trial).

3. Trial factor is barley cultivars and 20 barley cultivars grown for feed in our country will be included in the trial.

Process: In the current study, the conditions to be created by taking into account the water and temperature trials and the light and density values to be obtained from the preliminary studies will be used for the purpose of comparing barley varieties. The trial will be carried out according to the Random Plots Trial Design with 4 replications. Each growing tray (50x100x7 cm, 0.5 m²) will be considered as one parcel.

As a result of the trial, the details of which are given in the water application section; number of plants and fresh weight (g/m²), dry weight (g/m²) and leaf area (cm²) measurements, as well as RGR (mg/g.day), CGR (g/m².day), NAR (g/m². days) and LAR (cm²/g) calculations will be performed.

4. Within the scope of evaluations in terms of resource use and energy efficiency, while

calculating the water footprint, virtual water will be taken into account for all inputs in the construction phase of the prototype, as well as the daily water consumption of the system, and the water footprint of the prototype will be calculated annually.

While calculating the carbon footprint, the indirect and direct greenhouse gas consumption of the prototype will be taken into account, similar to the water footprint, and the calculation will be made by considering the study of Cheng et al., (2016).

The calculation of energy efficiency will be based on specific energy consumption (SET) and energy density, and these calculations will be carried out by taking into account the "Regulation on Increasing Efficiency in the Use of Energy Resources and Energy" published in the Official Gazette dated 27.10.2011 and numbered 28097 of the Ministry of Energy and Natural Resources. The following calculations will be made by considering each application factor separately for the energy consumed by the prototype during the whole production year:

Total Energy Consumption (TEP): It will be calculated on the basis of converting the total energy consumed during the entire production period into Kcal unit and dividing it by 107 and determining the TEP value.

Specific Energy Consumption (SET Mcal/ton): It will be obtained by dividing the annual amount of energy consumed by the amount of production.

Energy Intensity (Mcal/TL): The amount of energy consumed in order to produce one unit of economic value will be calculated according to the formula below:

$$\text{Enerji Yoğunluğu} = E/D$$

E = annual total energy consumption of the enterprise in toe.

$$D = (100/ Y_i\text{-PPI}) \times \sum (P_i \times F_i)$$

D = Economic value of annual production of goods in thousand (1000) Turkish Liras with prices for the year of manufacture.

$Y_i\text{-PPI}$ = Domestic producer price index of the relevant sector (To be determined according to the equivalent forage product prices).

P_i = Quantities of goods produced during the year.

F_i = In thousand (1000) Turkish Lira, the factory sales prices of the goods produced during the year (to be determined according to the equivalent roughage product prices).

5. Based on the data obtained from the prototype, the economic evaluations will be calculated according to the following parameters, over the values and costs to be obtained from the vertical farming system in 1 da during the 1-year production period. *Gross Production Value (GSÜD):* It will be found by adding the productive increases in plant capital to the value found by multiplying the amount of plant products obtained as a result of one-year production activities with the prices planned to be seized (Erkuş et al., 1995).

Gross Product (GSH): It will be obtained by adding the rent provision of the operating facilities to the Gross Production Value (GPP). "Pure Income" will be calculated by subtracting the operating costs from the gross product (Aydın and Unakıtan, 2016).

Total Operating Costs: Consisted of variable and fixed costs (Aydın and Unakıtan, 2016). Variable cost elements; seeds, chemicals, temporary labor, fuel, water fee, transportation-marketing and tool-machine repair-maintenance fee. fixed costs; Depreciation excluding variable operating costs, system repair and

maintenance, operator's wages, permanent workers' wages and taxes.

Gross Profit (TL): It will be calculated by subtracting variable costs from GSÜD.

Pure product (TL): It will be found by subtracting operating costs from GSH.

CONCLUSIONS

In general, a vertical farming facility to be established is expected to be affordable, operable, sustainable and reliable (Kalantari et al., 2017). In this context, when the researches, studies and inferences made in the literature are evaluated. Barley fodder, which will be produced as forage in the vertical farming system, has an significant potential in reducing the negative impact of agriculture on environment and ensuring food safety by using resources such as water and soil in the most efficient way (Yeşil and Tatar 2020). On the other hand, the efficiency and cost level of the system to be installed is directly related to the optimization of growing conditions such as temperature, humidity, seed frequency, water and energy requirements of barley fodder. Consequently, further studies on technical, environmental and economic aspects of the issue should be conducted.

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MICROLOCALITY EFFECTS ON AGROBIOLOGICAL CHARACTERISTICS OF PROKUPAC CV BT 1 AND BT 2 CLONES

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Abstract

The aim of the study is to show effect of ecological factors on agrobiological characteristics of two Prokupac clones BT 1 and BT 2 such as: developed vegetative mass, development of winter buds, number of developed clusters and yield as well as mechanical composition of grape berries and chemical characteristics of grape juice in microlocality of Pavlovci in Srem region, Serbia. For the purpose of the study 10 vines of both clones were examined separately. Average yield was higher for clone BT 2 (1.95 kg/vine) in comparison to clone BT 1 (1.76 kg/vine). Sugar content in grape must was greater for clone BT 2 (24.1%) in comparison to clone BT 1 (19.9%). Based on the results of Prokupac clones BT 1 and BT 2 in the locality of Pavlovci it can be concluded that clone BT 2 had greater average yield per vine as well as higher sugar content in berries and as such is a better clone for growing and producing high quality red wines.

Key words: Prokupac, clone, agrobiological characteristics, microlocality, grape

INTRODUCTION

Prokupac is the most widespread autochthonous grape variety in Serbia. Except in Serbia it is grown in North Macedonia and in smaller percent in Bulgaria and Russia. The variety is also known by synonyms: *Kameničarka*, *Rskavac*, *Nikodimka*, *Crnka*, *Propkupka*, *Zarčin*, *Skopsko crno*, *Majski čorni* etc.

It is characterized with strong vigor and yielding capacity. Top of young shoot is hairy and reddish-green. Ripe shoot is thick and reddish-brown with purple nodes, Leaf is large, parted in 3 with reddish nerves and leaf pedestal. Flower is hermaphrodite. Berries are medium large, round or slightly snippy with thick and dark blue epidermis. Prokupac bunch is medium large (150-300 g), with cylindrical or conical form, medium compact.

Ripening time occurs very late in the IV epoch. Average yield ranges from 12 to 18 t/ha; medium resistance to frost with winter buds freezing on temperatures below -18°C. Sensitive variety to *Plasmopara viticola*, medium resistant to *Uncinula necator*. Some Prokupac clones are characterized as highly resistant to *Botrytis cinerea*. Grape juice (must) can accumulate 18-25% of sugar content and 5-6 g/l of total acid content.

Prokupac is used for production of rose, high quality red wines and for coupage with other grape varieties (Žunić and Garić, 2017). As autochthonous grape variety Prokupac has great economic significance in Serbia. Long-term cultivation in diverse agro ecological conditions has caused the Prokupac to become a mixture of clones (genotypes). Polyclonal origin and the

accumulation of genetic mutations caused high variability within this cultivar.

Srem region it is important part of Serbia for grape growing and producing of high quality wines. By historic information one of the first vineyard in accient Serbia was planted in Srem region (Markovic, 2012).

According to an agricultural census from 2012 there were 2141.96 hectares of vineyards in Srem region out of which 1812.16 hectares had grape varieties meant for wine production (Table 1.). There are 2170 individual grape growers which accounts for 5.7% of agricultural activity in this region.

Table.1 Area under vineyards in Serbia and in Srem region

	Total (ha)	Wine varieties (ha)	Table varieties (ha)
Serbia	22.149,97	17.482,72	4.667,25
Srem	2.141,96	1.812,16	329.8
%	100	78.92	21.07

Clonal selection is an important method of determining plants that have better agrobiological or technological characteristics that other plants from the population group. Adequate choice of a clone is extremely important in the process of vineyard establishment.

Prokupac clonal selection is carried out in Faculty of Agriculture in Belgrade. Marković et al. (2008) has allocated 25 Prokupac clones which were tested for this study. These clones were subjected to laboratory testing for presence of damaging viruses, thus confirming the "natural virus free" status. Natural virus free clones can be used for further multiplication and

production of certified planting material. During the period of investigation of morphological characteristics of 25 clones, 13 clones were recognized by the Ministry of Agriculture as technologically better clones compared to standard variety. Clones BT 1 and BT 2 are currently in process of recognition and are being added to the sort list. The aim of this study was to determine the differences between clones BT 1 and BT 2 grown in Pavlovci locality in Srem region, Serbia, and also to determine how to they differ from the Prokupac population group.

MATERIALS AND METHODS

The study was done in an experimental vineyard at “Komuna” winery which is situated on the southern slopes of Fruška Gora mountain (GPS 45°5’45’’ latitude and 19°82’35’’ longitude). The vineyard was planted in 2014 and it consists of large number of wine and table cultivars and clones. Spacing between rows was 1.5 m and spacing between plants was 0.9 m. Trellis was constructed from wooden poles and wire in a way that enables optimal shoot, leaf and cluster distribution in space while also securing good insolation. The training system was “asymmetrical cordon” (Nakalamić, 1991) with trunk height of 100 cm. Short type pruning system with 3 spur buds was used.

For the purpose of the study 10 vines of both Prokupac clones BT 1 and BT 2 were examined separately. The clones were selected in Botunja village in southern Serbia, hence the “BT” abbreviation. Vegetative mass was determined by mass of pruned shoots on balance. Beginning and duration of the phenological stages were determined using BBCH by Lorenz et al., (1994). Fertility was determined by

interflorescens counting at the beginning and the end of flowering.

(pedicel) from each berry was carefully cut off with scissors so that as little mesocarp as possible was left on stem. Number of berries per bunch was also determined and berry mass per bunch and mass of stems were measured on analytical balance. From each clone 100 berries were selected for purpose of mechanical analysis and after measuring mass of berries, berry skin and seeds were separated. Mass of seeds and skin of 100 berries was measured on analytical balance, and number of seeds in 100 berries was determined by counting. Other parameters were obtained by computation.

Sugar content in the grape juice (must) was determined using Oeschle mostwage. The working principle of Oeschle mostwage is based on determining grape must density which is measured in Oeschle degrees (°Oe). Degrees Oeschle represent the difference between water density and must density on 4°C. Results of sugar content are shown in percents. The percent of sugar was determined using the following formula:

$$\% \text{ of sugar} = \text{°Oe} * 0.266 - 3$$

Total acid content was determined with titration method using 0.1M NaOH. The working principle of determining total acid content in grape must is based on neutralization of all acids with NaOH solution. Phenolphthalein was used as color indicator. The must was titrated with NaOH solution until colour changed from bright red to dark green. Total acid content was then determined by using the following formula:

$$\text{Total acid (g/l)} = \text{used NaOH} * F * 0.75$$

Mechanical composition of grape and berry was determined by Marković and Pržić method (2020). Bunches were measured for their weight, length and width, and rachis

F – normality factor of NaOH solution

Glicoacidometric index was determined as ratio of sugar content and total acid content.

RESULTS AND DISCUSSIONS

Climat condition of microlocality

Grapevine is grown in outside different conditions were numerous outside factors directly affecte growth and yield. Joint influence of enviromental factors on the grape vine properties is called grape vine ecology and it includes climate and pedologic conditions of the area where the grapevine is grown.

Climate factors of Srem region shown have been taken in the period of 30 years (1981-2010) and they can be considered representative for the study locality. Climate factors shown are: temperature, humidity and precipitation. Srem region climate is classified as temperate-continental.

Temperature data for the period 1981-2010 is as following: average annual temperature was 11.4°C, average vegetation temperature was 17.5°C. On average the coldest month was January (average minimal temperature of -3.6°C) and the warmest were July and August (average maximal monthly temperatures of 27.8°C and 27.9°C). The temperature results show that Srem region is very suitable for grape growing since the coldest period of the year is when grape vine is on dormant period when is most resistant to low temperatures and there is no significant risk of frost damage.

Average annual humidity for the analysed period was 76.42%. The most humid months were winter months (>80%) and the least humid were April and May (69% and 68%).

Srem wine region is generally favorable in terms of precipitation. High amount of precipitation in Jun has a positive impact on the phenophase of berry growth since the berries require a lot of water in that period.

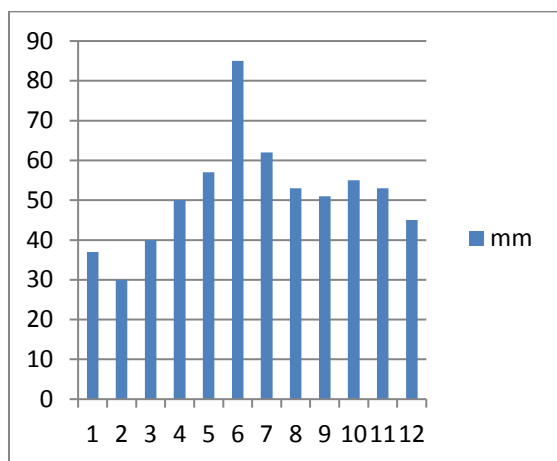


Figure 1. Average monthly precipitation for the period 1981-2010

The Fruška Gora locality is generally favourable for grape vine growing in terms of climate and pedologic conditions, especially because of proximity of the Danube river.

Agrobiological characteristics of BT 1 and BT 2 clones

Clone BT 1 had greater average mass of pruned shoots (291.5 g) compared to clone BT 2 (227.0 g), which means strong vigor and less developed inflorescences.

Phenological observations: bleeding started from 08.04. to 20.04. Bud bursting occurred on 24.4. without significant difference between the two clones. Growth of shoots started on 07.05 for clone BT 1 two days later, on 09.05. Clone BT 1 started

Average annual precipitation for the analyzed period was 620.3 mm. The month with the most average precipitation was Jun and the month with the least average precipitation was February (Figure 1.).

Low amount of precipitation in late summer and early autumn is also important because of its effect on berry ripening of cultivars whose berries ripening occurs very late such as Prokupac.

flowering earlier on 6.6. while clone BT 2 started flowering on 10.6. Harvest was carried out at full maturity on 14.10. Vegetation period represented by total number of days between bleeding phenophase and harvest was 189.

Table 2. Phenological observations of clones BT 1 and BT 2

Phenophase	Beginning/End	
Bleeding	08.04. – 20.04.	
Budburst	24.04.	
Growth of shoots	07.05. BT 1	09.05. BT 2
Flowering	06.06. BT 1	10.06. BT 2
Harvest	14.10.	
Number of days between bleeding and harvest = 189		

Average yield was higher for clone BT 2 (1.95 kg/vine) in comparison to clone BT 1 (1.76 kg/vine). Both clones have shown higher average yield per vine compared to plants from Prokupac population group (1.58 kg/vine).

Mechanical composition of clusters and berries was as following: clone BT 2 had greater average cluster mass (172.6 g) while

clone BT 1 had average clusters mass of (112.8 g); average number of berries in a cluster for clone BT 1 was (77.6) and for clone BT 2 (75.2); average berries mass in cluster was greater for clone BT 2 (165 g), average skin mass in 100 berries was higher for clone BT 2 (13.9 g); percent of berries in clusters and percent of mesocarp in berries was the same for both clones (96% and 92%); percent of berries skin was higher for clone BT 2 (5.5%) and percent of seeds in berries was higher for clone BT 1 (2.6%) (Table 1). This result are according to Markovic et al., 2017 and Markovic and Przic, 2018.

Table 3. Mechanical composition of berries of BT clones and Prokupac population plants

Clone	% of mesocarp	% of skin	% of seeds
BT 1	92	4.6	2.6
BT 2	92	5.5	1.8
PMpop	95.3	3	1.7

Bunch and berry structure are important characteristic of wine cultivars. Berry skin is important element of structure because it contains phenolic compounds which are extracted into wine, thus giving it color and odor (Lataief et al., 2006). Results of structural indices and percentage of berry skin, provides crucial information to a technologist in the process of maceration when making wine (Stoica et al., 2015). According to Zdunic et al., 2019; Zivkovic et al., 2016 and Downey et al., 2006 berry skin and mesocarp ratio in small sized berries is better in case of varieties with medium large and large berries. Increased ratio between pulp/berry skin causes “dillution“ of tanine and anthocyanin in juice.

During alcoholic fermentation of grape must complex biochemical processes are occurring as well as various physical and chemical changes which give wine as the result. As Prokupac is a variety for production of high quality red wines parameters such as sugar content, total acid content and pH of grape must are considered as very significant.

Sugar content in grape must was greater for clone BT 2 (24.1%) in comparison to clone BT 1 (19.9%); total acid content was for clone BT 2 6.7 g/l and for clone BT 1 6.1 g/l. Glycoacidometric index was higher for clone BT 2 (3.6) compared to clone BT 1 (3.2) and pH was also higher for clone BT 2 (3.29) compared to pH of clone BT 1 (3.19). This results are presented on Figure 2.

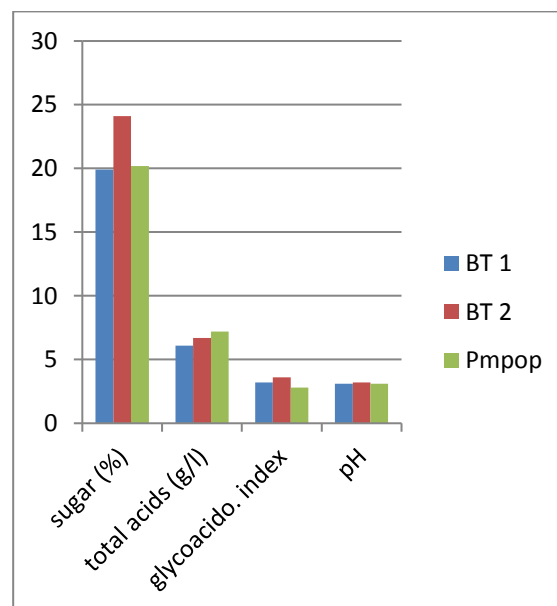


Figure 2. Chemical composition of grape must of BT clones and Prokupac population plants

CONCLUSIONS

Based on the analysis of ecological factors of the locality as well as agrobiological and technological properties of Prokupac clones BT 1 and BT 2 following conclusions can be made:

- Pavlovci locality is suitable for the growing of both clones;
- there were no significant differences in the starting of phenological phases between the clones except for the flowering – clone BT 1 started flowering 4 days earlier;
- clone BT 2 had greater average yield per vine;
- both clones have shown good mechanical composition of cluster and berries, clone BT 2 had greater percent of skin in the berries;
- clone BT 2 had greater content of sugar and total acid in grape must with higher enological potential;
- both clones have shown better agrobiological and technological characteristics than the plants from the Prokupac population group;
- clone BT 2 is the better clone for growing and for the production of high quality red wines.

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INFLUENCE OF AGROECOLOGICAL LOCALITIES CONDITIONS ON UVOLOGICAL AND TECHNOLOGICAL CHARACTERISTICS OF BAGRINA VARIETY

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Abstract

During the last few years, the reputation of less cultivated grape varieties has grown significantly. Today, Bagrina variety is not grown only in Serbia, but also in Serbia/Romania border zone, where it is found under the synonym Bragina, Bragina rosiu (Romania synonyms). Experiments have been set up in ampelographic collection of Institute of viticulture in Sremski Karlovci and on a privately-owned vineyard in village Arbanasce, Prokuplje. Bagrina were grafted on rootstock Kober 5BB. Vines were uniformly pruned (a two buds per spur and 12 buds per arc were left). Guyot training system was used. For the examined traits, mass of 100 berries (250.2 g), mass of the skin in 100 berries (10.38 g) and mass of seeds of 100 berries (5.90) were found to be higher for Sremski Karlovci than in Prokuplje. A strong correlation was found between percent of berry skin and percent of seed mass in berry. The higher sugar content in grape juice was determined in grape from Sremski Karlovci locality (20.6%) and lower value for Prokuplje locality (19.8%). Based on the in grape juice, In grape juice Bagrina had a lower content of total organic acids (4.73 g/l) in Prokuplje. Based on results of mechanical composition of berry and chemical composition of grape juice it can conclude that Bagrina at Sremski Karlovci locality showed better results compared to other localities, and can be recommended for growing in these and other localities with similar or same agroecological conditions.

Key words: *Bagrina, locality, uvological and technological characteristics, grape, quality*

INTRODUCTION

Which cultivars from the present day are the oldest? Today, insights into filiations of cultivars provide genetic fingerprints, a technique corresponding to archaeological investigation.

These neglected cultivars, representing witnesses of the variety assortment of former centuries, are highly endangered. The agronomic and technological features of rare and minor cultivars are often unknown. Most are grown in vineyards that are over 80 years old, maintained by elderly people, mainly in mixed plantings. Continued maintenance is often doubtful. In addition, most neglected traditional cultivars are not listed in national catalogs.

Thus, they cannot be legally grown. Efforts to facilitate the registration of neglected and endangered traditional grapevine cultivars have failed until now. Most were abandoned in the past because of deficiencies such as late ripening, high acidity, overcropping, bad fruit set or lack of color, the better features of the offspring, or the arrival of migrating cultivars. Their aptitudes and value for both vine-growers and wine drinkers are best evaluated under experimental conditions “on-farm” (Maul et al, 2019).

Indigenous and old domesticated grape varieties dominated the assortment from the pre-phylloxera period until the renewal of viticulture after the Second World War. A

small number of them are still grown in private vineyards. Most indigenous varieties are lost forever. In the late 1980s and 1990s, prospecting started in several European countries to collect and preserve traditional cultivars and their respective clonal diversity (Costacurta, 1991; Boursiquot, 1998; Maigre et al., 1999).

Fortunately, in the last 20 years, the reputation of minor grape cultivars has increased considerably. Growers' magazines and wine journals have reported the renaissance of neglected and even threatened grape cultivars, raising awareness and promoting their cultivation and consumption. To avoid the disappearance of this unique germplasm, and to preserve this cultural heritage, conservation efforts and lobbying for their survival are essential. This study aimed to determine the production and biological value of old domestic and domesticated grape varieties to confirm the possibility of their return to the assortment of Fruška Gora and Prokuplje. Another aim of this paper was to determine technological characteristics of the rarely represented variety Bagrina and to confirm the possibility of its return to the actual grapevine assortment of Serbian, as well as to give recommendations for following breeding programs.

MATERIAL AND METHODS

Bagrina cultivar is an old Balkan grapevine intended for high-quality, white wine production (Milutinović et al., 2000). Today, the Bagrina variety is not grown only in Serbia, but also in Serbia/Romania border zone, where it is found under the synonym Bragina, Bagrina krajinska (Serbia), Bragina rara, Bragina rosiu (Romania synonyms). This variety originates from Romania, from where it was

transferred to our country. It is mostly grown in Romania and somewhat less in Bulgaria and others. In our country, it is grown only in the Timok region. It belongs to Proles pontica (*Convarietas pontica*). At „Braghina“ grape variety the cross-section of the woody shoot is circular, the structure of the surface is striated, brown-yellow, the lenticels are absent, the erect hairs on nodes and internodes are absent, the length of internode is short and the diameter of internodes is small (Gorjan Sergiu, 2011). The flower is morphologically hermaphroditic, and functionally female-with five curved anthers in which sterile pollen is formed. Because it has a functional female flower, fruitfulness is irregular because the fruit set is low and loose bunch often occur. That is why this cultivar should be cultured in vineyards in which other cultivars served as pollinizers (Cindrić et al., 1994). Author Žunić et al. (2017) state that fertilization in this variety is irregular and that good pollinators for this variety are: Prokupac, Smederevka, and Plovdina. While the group of authors Milutinović et al. (2000) state that the varieties Chardonnay, Smederevka, and SV 12-375 are also good pollinators of Bagrina. The bunch is medium-sized or large, branched, medium-dense, and most often loose and loose. Observing the phenological side of this variety, it matures in 3 epochs, as a late variety. Although according to long-term research it belongs to the varieties with the highest harvest date variability (Ruml et al., 2013). The Bagrina variety is the subject of examination of this scientific research by the results in which it showed significant uvological and technological characteristics, but also genetic variability. The population contains multiple clones

and variations. Two clones are known, a clone with a whole leaf and a clone with an incised multi-part leaf, but clones were also observed according to the intensity of skin color. A clone with a multi-part leaf is more profitable and economical for growing and producing grapes and wine. Based on the experience of the producers, they state that clones with more intense coloration had a higher content of anthocyanins in the skin and produced more aromatic and complete wines. Attempts are being made to create Bagrina with a morphological and functionally hermaphroditic flower.

The tests were performed in the experimental field of the Institute of Viticulture, Fruit Growing and Horticulture, Faculty of Agriculture in Novi Sad. It is located in the northern part of the Fruška Gora vineyards in Sremski Karlovci, and several some of autochthonous and old domesticated varieties are kept and examined. The second part of the examination is located on a private plantation in the village of Arbanasce, in the municipality of Prokuplje, in the Toplica region. The paper presents the one-year (2021) results of research on one wine variety Bagrina. Both vineyards that are the subject of this paper were planted with a spacing of 3 x 1 m and grown with the Simple Guyot system. For examination of structural indicators of berry and chemical

content of grape juice were selected 10 vines. The examined structural indicators were, as follows: the weight of 100 berries, the weight of skin in 100 berries, the weight of seeds in 100 berries, the weight of 100 seeds and number of seeds in 100 berries, the length and width of berries. The chemical composition of grape juice was presented through sugar % (refractometer - Pocket Atago Pal 1), total organic acid content g/l (titration with n/4 NaOH), and pH value.

RESULTS AND DISCUSSION

The production of quality grapes and wine depends on the relationship of various factors such as: locality, variety and applied agro and ampelotechnical measures (Ranković-Vasić et al., 2022). According to the author Žunić et al. (2017), the yield of Bagrine grapes ranges from 3,000 to 18,000 kg/ha. The general impression of the producers of this variety is that it is a high-yielding variety and that it is necessary to regulate its yield during hinges and during the ripening period. The results of the research on the quality of yield of the Bagrina variety are given in Tables 1 and 2, which were conducted during the 2021 growing season. Table 1 shows that the structural indicators of berries for the variety Bagrina for the localities Sremski Karlovci and Prokuplje

Table 1. Structural indicators of berry variety Bagrina.

Localitet	Weight of 100 berries	Weight of skin in 100 berries	Weight of seeds in 100 berries	Weight of 100 seeds	Number of seeds in 100 berries	Length of berries	Width of berries
Sremski Karlovci	250	10,3752	5,9057	3.2310	182	15,34	13,54
Prokuplje	201	5,8707	4,8467	2.5134	196	15,90	12,87

For the examined traits, the mass of 100 berries (250.2 g), the mass of the skin in 100 berries (10.38 g), and the mass of seeds of 100 berries (5.91) were found to be higher in Sremski Karlovci than in Prokuplje, where lower values were found for the same properties. Significant differences were found when examining the characteristics of the number of seeds in 100 berries, with a higher value (196) at the site in Prokuplje.

A strong correlation was found between the share of skin in the berry and the share of seed mass in the berry.

Žunić et. al. (2017) notes that the Bagrina variety exhibits good traits for uvological characteristics. The weight of the bunch ranges from 90.00 to 200.00 g, while the number of berries per bunch is 40-80, and the weight of the berries is 70.00-170.00 g.

Table 2. Chemical content of grape juice in variety Bagrina.

Localitet	Sugar content	Total acids content	pH value
Sremski Karlovci	20.6	6.49	3.49
Prokuplje	19.8	4.73	3.36

The higher sugar content in the wider area was determined at the locality in Sremski Karlovci (20.6%), while the lower value was determined at the locality in Prokuplje (19.8%). Based on the content of total organic acids in grape juice, the Bagrina variety had a lower value (4.73 g/l) at the site in Prokuplje. According to the authors Žunić et al. (2017) the more important technological and orthanoleptic characteristics of the Bagrina variety are indicated. Shira in the Bagrina variety is colorless, has a pleasant taste and smell, and contains 18-23% sugar and 5-8 g / l of total acids.

CONCLUSION

Minor cultivars represent huge value concerning cultural heritage, adaptation to ecological conditions, diversification of products and marketing of specialties with higher added value. Bagrina cultivar is an old Balkan grapevine intended for high-quality, white wine production. For the examined traits, the mass of 100 berries (250.2 g), the mass of the skin in 100 berries

(10.38g) and mass of seeds of 100 berries (5.90) were found to be higher for Sremski Karlovci than in Prokuplje. A strong correlation was found between the percent of berry skin and the percent of seed mass in berry. The higher sugar content in rape juice was determined in grapes from Sremski Karlovci locality (20.6%) and lower value for Prokuplje locality (19.8%).Based on results of mechanical composition of berry and chemical composition of grape juice it can conclude that Bagrina at Sremski Karlovci locality showed better results compared to other localities, and can be recommended for growing in these and other localities with similar or same agroecological conditions.

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ROOTSTOCKS INFLUENCE ON AGROBIOLOGICAL AND TECHNOLOGICAL CHARACTERISTICS OF TWO CABERNET SAUVIGNON CV CLONES

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Abstract

The aim of this study was to determine differences between Cabernet sauvignon clones 191 and 412 under the influence of rootstock 101-14. During the research period it was determined duration of phenological stages, vegetative and fertility potential, yield, grape and berries mechanical composition and qualitative parameters of grape juice and wine. Clone 191 had the average shoot growth of 130.62 cm while clone 412 had average shoot growth of 144.30 cm. Based on number of developed inflorescences and bunches yield of clone 191 varied from 1.754 to 2.158 kg per vine and for clone 412 varied from 1.041 to 1.784 kg per vine. In terms of grape and berries mechanical composition clone 191 stood out in most characteristics compared to clone 412. Clone 191 had a higher sugar content (25.09%) and clone 412 had a higher content of total acids (7.6 g/l) in must. Total acids in wine were higher for clone 412 (7.13 g/l) and clone 191 had higher alcohol content in wine (14.4% vol.). In sensory evolution clone 412 had 19.1 points and clone 191 had 17.2 points.

Key words: Cabernet Sauvignon, clone, berries, mechanical characteristics, win.

INTRODUCTION

Cabernet Sauvignon is one of the most common varieties in the world. It is a very old variety originating from Bordeaux, France, that is cultivated in most countries and produces high quality wines (Gil and Pszczółkowski, 2007). It is a very adaptable variety to different types of soil and climate conditions which has enabled its distribution around the world (Robinson, 2006). In the total wine assortment ranks second with a total of 341.000 hectares, or 4% of the world's area under vineyards (OIV, 2017). The use of rootstocks has become a compulsory practice in commercial grapevine plantations, mainly because it provides resistance to root pests, adaptation to different soil types, and an increase in vine productivity and grape quality (Marguerit, 2012). The vine rootstock affects the varieties in different ways such as in vine physiology, yield components, vigor, fruitfulness, berry size and phenology.

However, the vigor induced by the rootstock on scion is the factor most often considered by growers in choosing a rootstock (Winkler et al., 1974). Agrobiological characteristics of Cabernet Sauvignon clones depends on the agrobiological conditions of the region, application of ampelotechnics, rootstock, training system and method of pruning (Brighenti et al., 2010).

MATERIALS AND METHODS

In this research were used two clones 191 and 412 of Cabernet Sauvignon variety that were grafted on rootstock 101-14. Planting distance was 2.3 x 0.8 m, training system-Royal cordon and with applied short type pruning. Duration of phenological stages was determined by Lorenz et al., (1994). Shoots growth measuring was done in three repetitions on 15 days interval. Fertility was determined by inflorescences counting at the beginning and

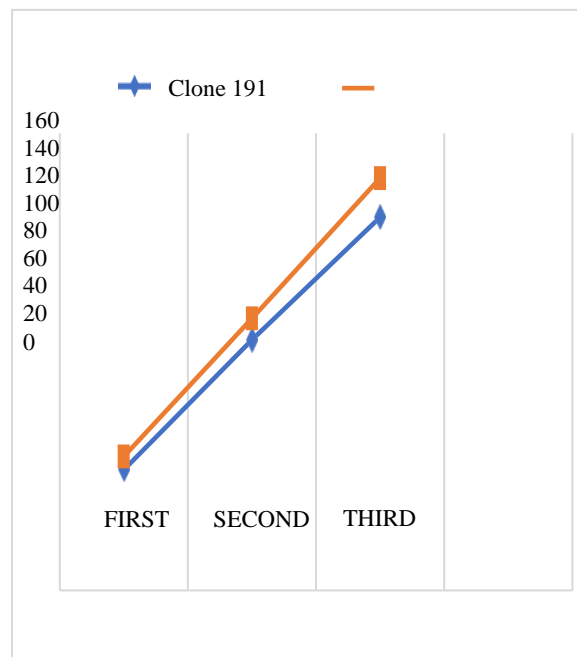
end of flowering. Grape samples of investigated clones were collected and their analysis was performed at laboratory of Faculty of Agriculture University of Belgrade. Mechanical composition of grape and berry was determined by Marković and Pržić method (2020). Bunches were measured for their weight, length and width, and rachis (pedicel) from each berry was carefully cut off with scissors so that as little mesocarp as possible was left on stem. Number of berries per bunch was also determined and berry mass per bunch and mass of stems were measured on analytical balance. From each clone 100 berries were selected for purpose of mechanical analysis and after measuring mass of berries, berry skin and seeds were separated. Mass of seeds and skin of 100 berries was measured on analytical balance, and number of seeds in 100 berries was determined by counting. Other parameters were obtained by computation.

Sugar content of grape must was analyzed using Oechsle mostwage, total acid content was determined by titration method using 0.1 M NaOH and pH was determined with pH meter. Physico-chemical wine analysis was done by standard OIV methods. For sensor vine evaluation was used scale up to 20 points (2 points for color, 2 points for clarity, 8 points for fragrance and 8 points for taste).

RESULTS AND DISCUSSIONS

In phenological observation for both clones was founded that bleeding started at the same time on 24.05. From veraison to full maturity passed 62 days. The number of days from bleeding to harvest lasted 192 days (Table 1). The duration of phenological phenophases was shorter in the examined clones compared to the Przic (2014) study of Cabernet Sauvignon variety, where it lasted 217 days.

Table 1.
Phenological observation of clones 191 and 412



Phenophases	Beginning	Termination	Duration (days)
Bleeding	24.03.	10.04.	18
Bud burst	13.04.	21.04.	9
Growth of shoots	28.04.		
Flowering	29.05.	09.06.	12
Berry growth	15.06.		
Maturation	Veraison	01.08.	62
	Grape harvest (full maturity)	01.10.	
Number of days from bleeding to harvest			192

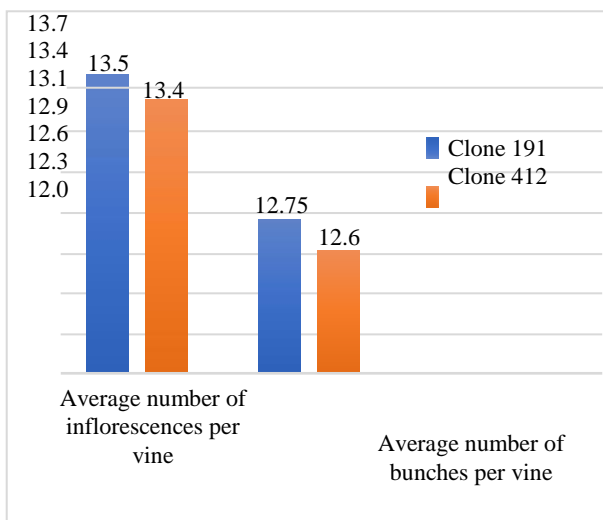
In the first measurement clone 191 had an average shoot growth of 42.24 cm, in second 87.72 and in the third 130.62 cm and clone 412 in the first measurement had an average shoot growth of 46.87 cm, in second 95.12 and in the third 144.30 cm. Statistical analysis showed that there was a significant difference in the average values of shoot growth between clones 191 and 412 of the Cabernet Sauvignon variety in all three measurements (Graph 1). Examinations by Marković (2001) and Przic (2014) showed that Cabernet Sauvignon shows variability in the growth of shoots depending on the vine rootstock on which it is grafted. On

those vine rootstocks, there was a higher increase in shoots that were grafted on the vine rootstock 101-14.

In term of fertility, statistical analyses showed that there was not a significant difference in the average values in number of inflorescences and bunches per vine, but there was significant difference in terms of average yield per vine between clones 191 and 412 Cabernet Sauvignon variety.

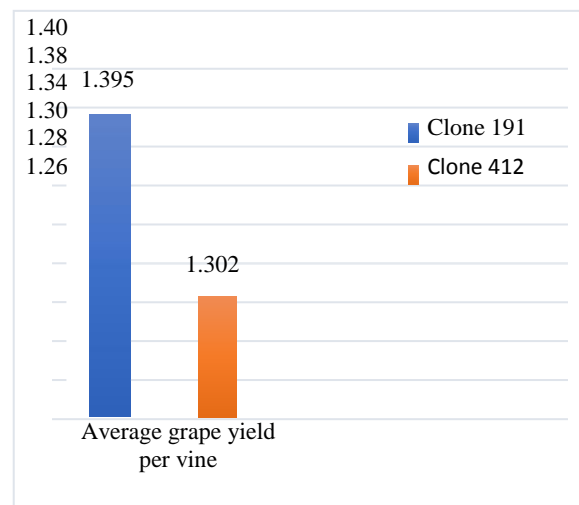
Graph 1. Shoots growth of clones 191 and 412

Clone 191 in average had 13.5 differentiated inflorescences and 13.4 Clone 412 developed bunches per vine compared to clone 412 which had in average 12.75 differentiated inflorescences and 12.6 developed bunches per vine (Graph 2).



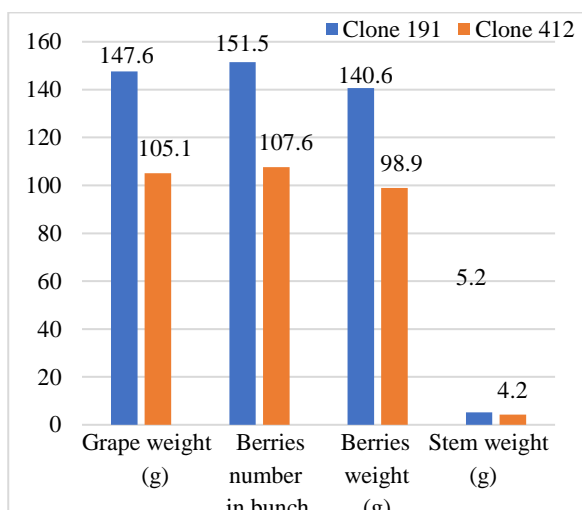
Graph 2. Average number of inflorescences and bunches per vine of clone 191 and 412

Average grape yield for clone 191 was 1.395 kg per vine and for clone 412 was 1.302 kg per vine (Graph 3). Yield of clone 191 varied from 1.754 to 2.158 kg per vine and yield for clone 412 varied from 1.041 to 1.784 kg per vine. In the study of Marković et al., (2011) Cabernet Sauvignon clones ISV-F-V5 and ISV-F-V6 had a higher number of developed inflorescences, clusters and yields per vine compared to the tested clones.

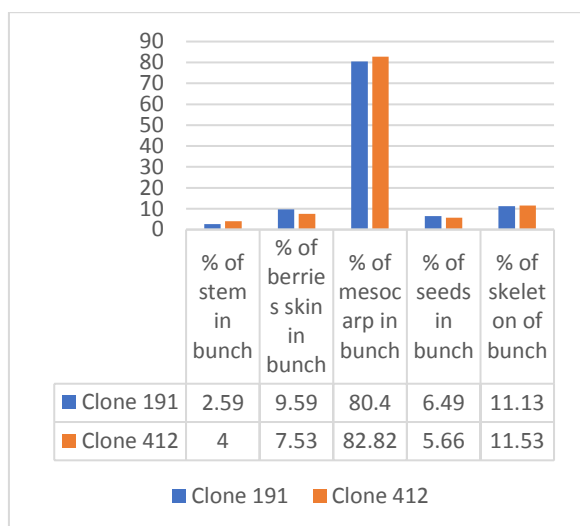


Graph 3. Average grape yield per vine of clones 191 and 412

In research of mechanical and uvometric characteristics of berries and bunches were founded higher bunch weight (147.6 g), berries number in bunch (151.5 g), berries weight (140.6 g), stem weigh (5.2 g), percent of berries skin in bunch (9.59%), percent of seeds in bunch (6.49%) and skeleton bunch (13.11%) for clone 191 compared to clone 412 which showed a higher percent of stem in bunch (4%) and percent of mesocarp in bunch (82.82%). Rersults were showed on Graph 4 and 5. Compared to clones ISV-F-V5 and ISV-F-V6 of Cabernet Sauvignon variety examined by Markovic et al., (2011) clones 191 and 412 had for lurge number of parameters smaller value of uvometric characteristics.



Graph 4. Average values of bunch composition of clones 191 and 412



Graph 5. Bunch structure of clones 191 and 412

Sugar content in grape juice (must) was higher for clone 191 (25.09%) compared to clone 412 (23.76%). Clone 412 had a higher content of total acids (7.6 g/l) relative to clone 191 (6.8%). Clone 191 had glycoacidometric index 3.7 and clone 412 had glycoacidometric index 3.1. For clone 191 pH was higher (3.19) compared to clone 412 (3.03), Results are shown in Table 2.

Table 2. Chemical composition of juice (must) for clones 191 and 412

Clones	Sugar content (%)	Content of total acids (g/l)	Glycoacidometric index	pH
191	25.09	6.8	3.7	3.19
412	23.76	7.6	3.1	3.03

Wine of clone 191 had higher content of free SO₂ (8.96 mg/l) and total SO₂ (18.25 mg/l) compared to clone 412 which had 6.4 mg/l content of free SO₂ and total SO₂ 18.25 mg/l. Specific weight was about the same between clone 191 (0.9929 g/cm³) and clone 412 (0.9927 g/cm³). Total acids in wine were lower for clone 191 (6.38%) and higher for clone 412 (7.13 g/l). In clone 191 pH was higher (3.19) compared to clone 412 (2.29). Clone 191 had 14.4% vol. alcohol content in wine and clone 412 had 13.9% vol. (Table 3).

Table 3. Physicochemical composition of wine of clones 191 and 412

Parameters	Clone 191	Clone 412
Free SO ₂ (mg/l)	8.96	6.4
Total SO ₂ (mg/l)	18.97	18.25
Specific weight g/cm ³	0.9929	0.9927
Total acids (g/l)	6.38	7.13
pH	3.19	2.29
Alcohol (%)	14.4	13.9

And finally in sensory evaluation of wine, both clones were rated with 2 points for color and clarity, while clone 191 had a lower rating (6 points) on fragrance compared to clone 412 (7.3 points), as well as a lower rating on taste

(7.2 point) compared to clone 412 (7.8 points). Clone 412 had higher overall rating of 19.1 points, and clone 191 had an overall rating on 17.2 points out of maximum of 20 (Table 4).

Table 4. Sensory evaluation of wine of clone 191 and 412

Parameters	Clone 191	Clone 412
Color	2	2
Clarity	2	2
Fragrance	6	7.3
Taste	7.2	7.8
Overall rating	17.2	19.1

The wine obtained from clone 191 is a relatively young, no aged wine, so the color is more intense than in other wines. The smell is typical. The taste is slightly strong due to the feeling of pronounced acids. The wine has sensory characteristics typical of young Cabernet Sauvignon and the wine from clone 412 has a more pronounced color compared to clone 191, with a very pleasant aroma. The scent of violet is dominant and wine taste pleasant and harmonious. Presented results are according to Przic and Markovic 2019.

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CONCLUSIONS

Based on the influence of rootstock 101-14 on agrobiological and technological characteristics of Cabernet Sauvignon clones 191 and 412 it can be concluded that:

- both clones had the same duration of phenological phenophases.
- the higher increase in shoots had clone 412 compared to clone 191.

- clone 191 stood out in regard to the potential yield capacity compared to clone 412.
- both clones had good mechanical structure of berries and bunches.
- in grape juice (must) clone 191 had a higher sugar content compared to clone 412, while the clone 412 had a higher content of total acids in grape juice-must compared to clone 191.
- in terms of physico-chemical analysis and sensory evaluation, clone 412 is better clone for production of high quality wines.

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INFLUENCE OF GRAPE MATURITY ON CONTENT OF SOME PHENOLIC COMPOUNDS IN WINE

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Abstract

*In recent years, phenolics are of special interest, due to their beneficial effects for human health. Furthermore, phenolic compounds are extremely important constituents of grapes because of the quality parameters to which they contribute greatly. They include the nonflavonoids (hydroxybenzoic and hydroxycinnamic acids and stilbenes) and flavonoids (anthocyanins, flavan-3-ols and flavonols). Anthocyanins are responsible for the colour of red grapes. Flavan-3-ols are responsible for the adstringent taste sensation of grapes. Different factors such as phenophases of ripening, irrigation, soil type, and topography exert positive or negative effects on the content and composition of certain grape polyphenols. The aim of this work was to study how grape maturity affects on content of different phenolic compounds in wine. Grapes variety Cabernet Sauvignon were harvested in three different stages of maturity: vèraison, optimal enological maturity and overripeness, which originated from vineyards belonging to experimental field "Radmilovac" of the Faculty of Agriculture in Zemun, University of Belgrade (Serbia). The grapes of vèraison stage were harvested at last week of August. After this stage grapes were harvested at optimal enological maturity at first week of October. Three weeks after optimal enological maturity, grapes were harvested at overripeness stage. Grapes crashing and destemming were followed by sulfitation with 10 g of K₂S₂O₅ per 100 kg and inoculation of yeast strain *Saccharomyces cerevisiae* in the amount of 20 g/hl (BDX, Lallemand, Canada). Alcohol fermentation with maceration lasted 21 days at temperature of 25±3° C using the "pigeage" system (mechanically punching down). After that pomace was separated and obtained wine samples were bottled and stored until analyses. Caffeic acid, gallic acid and trans-resveratrol were quantified using LC-MS/MS system (Agilent LCTQ 6495C Triple Quadrupole). Prior to analyses, wine samples were prepared by solid phase extraction (SPE). The highest concentration of gallic acid (5.18±0.20 mg/l) and trans-resveratrol (0.58±0.04 mg/l) were measured in wine derived of grapes which were at first stage of maturation (vèraison). Gallic acid was least present in overripe grape wine and it was 1.59±0.15 mg/l. Caffeic acid was reached the highest value in grape wine of optimal enological maturity and after that was observed low concentration in overripe grape wine. There was no statistical significant difference in content of analyzed phenolic compounds in wine samples that derived from grapes at different maturation stages.*

Key words: - phenolic compounds, grape ripening, veraison, wine

INTRODUCTION

Phenolic compounds are divided into two groups: flavonoid (anthocyanins, flavan-3-ols, condensed tannins and flavonols) and non-flavonoid compounds (phenolic acids and stilbenes). The composition of each family of polyphenols is directly responsible for the special characteristics of specific grape varieties and of the resulting wine (Anđelković et al.,

2013). Many compounds of enological interest (phenolic compounds, aroma precursors) are known to be accumulated in that tissue throughout ripening (Vicens et al., 2009). Vèraison is the period when grape berries go through several changes i.e. the green color is changing into yellow-green for the white grapes or into red and different blue nuances for the red grapes due to accumulation of anthocyanins in

the skins. During véraison the grapes accumulate color (anthocyanins for red grapes), aroma compounds, tannins and minerals. The moment when the grape is fully ripe, called physiological ripeness, is characterized by maximum sugar content, and it is also the moment when the sugar, acidity and pH levels are in a good balance for harvesting. At the time of the late harvest the berries are naturally dehydrated, associated with shrinkage of the berries (Ivanova et al., 2011). Grape maturity is important to the overall quality of red wine, so it is important to know how content of certain phenolic compounds changes during this period. Furthermore, the aim of this study was to determine optimal harvesting period for the highest content of gallic acid, caffeic acid and *trans*-resveratrol in derived wine.

MATERIALS AND METHODS

The grape variety Cabernet Sauvignon was harvested at three different stages of maturity: véraison, optimal enological maturity and overripeness, which originated from vineyards belonging to experimental field "Radmilovac" of the Faculty of Agriculture, University of Belgrade. After grapes crashing and destemming, the samples were sulfited with 10 g of $K_2S_2O_5$ per 100 kg and yeast strain *Saccharomyces cerevisiae* in the amount of 20 g/hl (BDX, Lallemand, Canada) were inoculated. The grapes of véraison stage were harvested at last week of August. Total sugar content was 19.6 % and titratable acidity by titration with NaOH, was 7.5 g/l eqv. tartaric acid. Total sugar content was corrected to 24% by adding sugar (saccharose). After this stage grapes were harvested at optimal enological maturity at first week of October. Total sugar content was 23.5 % and titratable acidity by titration with NaOH, was 6.8 g/l eqv. tartaric acid. Three weeks after optimal enological maturity, grapes were harvested at overripeness stage, with the highest sugar content (25.1 %)

and titratable acidity 4.6 g/l eqv. tartaric acid. Alcohol fermentation with maceration lasted 21 day at temperature of $25\pm 3^\circ C$ using the "pigeage" system (mechanically punching down). After that pomace was separated and obtained wine samples were bottled and stored until analyses. Caffeic acid, gallic acid and *trans*-resveratrol were quantified using a LC-MS/MS system (Agilent LCTQ 6495C Triple Quadrupole). Prior to analyses, wine samples were prepared by solid phase extraction (SPE).

RESULTS AND DISCUSSIONS

The highest concentration of gallic acid (5.18 ± 0.20 mg/l) and *trans*-resveratrol (0.58 ± 0.04 mg/l) were measured in wine derived of grapes from véraison stage (Figure 1 and Figure 3). Gallic acid was least present in overripe grape wine and it was 1.59 ± 0.15 mg/l (Figure 1) The literature has showed that the highest content of gallic acid was identified in grape seeds of véraison stage (de Simón et al., 1992). There was no statistical significant difference in content of analyzed phenolic compounds in wine samples that derived from grapes at different maturation stages.

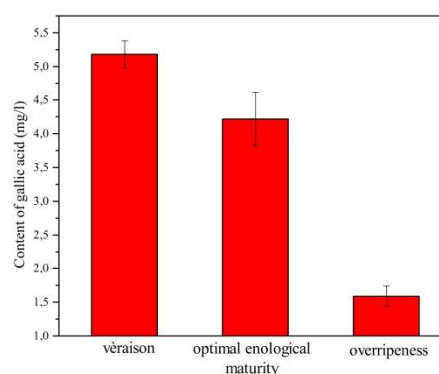


Figure 1. Content of gallic acid in wine from grapes of different stages of maturity

Caffeic acid was reached the highest value in grape wine of optimal enological maturity and after that was observed low concentration in overripe grape wine (Figure 2). According to Ivanova et al. (2011), a such decrease can be explained by changes in the concentration of

hydroxycinnamic acid esters in the skin of grapes that can be oxidized, build complexes with other compounds or participate in the synthesis of acetylated anthocyanins.

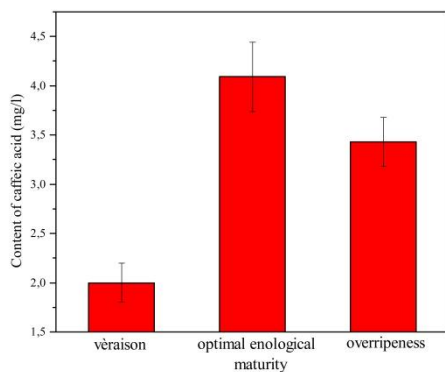


Figure 2. Content of caffeic acid in wine from grapes of different stages of maturity

According to Geana et al. (2015), who analysed five different grapes varieties (Pinot noir, Mamaia, Cabernet sauvignon, Fetească neagră,

CONCLUSIONS

The highest content of *trans*-resveratrol and gallic acid was obtained in wine from grape at véraison stage. For the highest content of caffeic acid in wine it was necessary full riped grape. However, there was no statistical significant difference in content of analyzed phenolic compounds in wine samples that derived from grapes of different maturity.

ACKNOWLEDGEMENTS

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Merlot) at different stages of ripening, have concluded that content of *trans*-resveratrol depends on grape variety. Grape variety Pinot noir showed the more ripe the grapes the lower content of *trans*-resveratrol will be identified what is in agreement with our study (Geana et al., 2015).

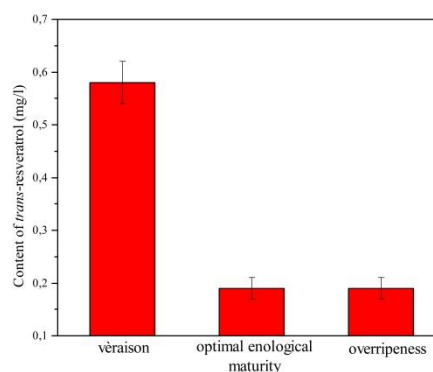


Figure 3. Content of *trans*-resveratrol in wine from grapes of different stages of maturity

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THE POSSIBILITY OF DELAYED FRUIT THINNING IN GALA APPLE

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Abstract

The optimal period of apple fruit thinning is from the fruit set until the moment when diameter of the king fruit is 15 mm. After that period, the reaction of chemical agents is weaker. The aim of this study was to achieve chemical thinning of apple fruits with larger diameter by increasing concentrations of met amitron, ethephon, 6-benzyladenine and α -naphthylacetic acid compared to those of recommended. The following treatments were applied: 1) met amitron 250 mg/l (MM250), 2) met amitron 250 mg/l + 6-benzyladenine 150 mg/l (MM250+BA150), met amitron 250 mg/l + ethephon 300 mg/l (MM250+E300), 6-benzyladenine 150 mg/l + α -naphthylacetic acid 15 mg/l + ethephon 300 mg/l (BA150+NAA15+E300), α -naphthylacetic acid 15 mg/l + ethephon 300 mg/l (NAA15+E300), ethephon 300 mg/l (E300) and commercial treatment (CT). The treatments were applied 28 days after full bloom when average diameter of king fruit was 19 mm. Results show that the treatments E300 + BA150 + NAA15, E300 + NAA15 and MM250 + E300 significantly reduced the number of fruits compared to the CT. The yield per tree of these treatments was almost equal to the CT, however the significantly larger fruits than in the CT were determined in the treatments E300 + BA150 + NAA15 and E300 + NAA15. Fruit firmness had lower values, whereas starch index had higher values in the treatments with ethephon, while the statistically significant difference compared to the CT was found only in the treatment when ethephon was applied alone. There were no significant differences among the treatments in terms of soluble solids and total acid content.

Key words: *apple, met amitron, ethephon, yield, fruit quality*

INTRODUCTION

Apple has become a very important fruit species in Serbia. According to FAOSTAT, the average annual production of apples in Serbia for the period from 2016 to 2020 was 445,706 tons. In modern apple orchards, yield of up to 80 tons per hectare are achieved in some years.

Apple trees usually produce much more flower clusters than they need for obtaining high quality and regular marketable crops. If the fruit set is too high quality of fruits is low, diameter of fruit is below market demand and biennial bearing is expressed. Fruit thinning is an important technique in apple growing that reduce the number of fruits per plant and achieve the required fruit size and quality (Gonzalez, 2019).

The main goal in modern apple production is to achieve high and regular yields and good fruit quality. Thinning of flowers or

fruits improves the quality of fruits and formation of buds for the next year, and this method has become a standard procedure in the production of many fruit species (Wertheim, 1998). Apple cultivars have a wide range of biennial bearing response ranging from severe to somewhat minor fluctuations. However, nearly all apple cultivars can benefit from some level of thinning (Greene and Costa, 2013). Manual thinning of apple fruits is often not feasible due to limited labor and high cost. Therefore, it is necessary to apply fruit chemical thinning in order to ensure profitable apple production (Stopar, 2007). Chemical thinning response depends on many factors such as treated variety, the type and concentration of the chemical thinned used, the environmental factors during and after application, tree factors, the timing of application, etc. (Stopar et al., 2007).

Ethephon has long been used as a thinning agent. Its main advantages are that it greatly improves the formation of flowers for the next year and can be applied over a longer period of time (Stopar, 2007). It is effective of average fruit diameter between 16 and 22 mm (Marini, 1996). Because larger fruits are more sensitive to ethephon, unlike most other chemical thinning compounds, ethephon can be used as a means of later fruit thinning and is also called a "last chance" agent, where other chemical thinners do not work, or where the manufacturer made an erroneous assessment and failed to apply at an adequate time (Greene, 2002).

Metamitron is the photosynthetic inhibitor, originally herbicide functioning through inhibition of photosystem II where blocking the electron transport and chlorophyll fluorescence is enhanced (Dorigoni and Lezzer, 2007; Basak, 2011; Stopar, 2017). It exhibited thinning activity when applied to apple fruitlets at the 10-12 mm of diameter stage (Basak, 2011), or even later at 20 mm (McArtney and Obermiller, 2012; Greene and Costa, 2013). Metamitron is applied at a concentration ranging from 1.1 up to 2.2 kg ha⁻¹ in single or repeated applications (Costa et al., 2018).

Naphtylacetic acid (NAA) is probably the most reliable postbloom thinner and is generally used on cultivars that are "difficult to thin" (Costa et al., 2018). The concentrations normally used range between 2 and 20 mgL⁻¹ applied when king fruit cross-sectional diameter is 4-6 mm (Costa et al., 2018). Lower concentrations may cause insufficient thinning, while higher concentrations may cause the formation of pygmy fruits (Marini, 1996). NAA applied at this stage is less aggressive, and the risk of overthinning is strongly reduced. It also could be used when diameter of central fruit is 10-12 mm (Radivojević, 2020). The plant growth regulator 6-benzyladenine (BA) became one of the most used commercial postbloom thinners for pome fruit at the

beginning of this century (Fallahi and Greene, 2010). The active ingredient is 6-benzyl amino purin which is normally applied at the concentration between 50 and 150 mgL⁻¹ when the diameter of the king fruit reaches 10-14 mm (Costa et al., 2018). The aim of this study was to study the possibility of chemical thinning of apple fruits with larger diameter, which are already treated in a regular thinning window.

MATERIALS AND METHODS

The experiment was conducted during 2021 on mature 'Gala' apple trees in commercial orchard located at the slopes of mountain Fruška Gora (45° 6' N, 19° 46' E, altitude 260 m). The trees were 3.0 m high, and spaced at 3.2 x 0.8 m (3,906 trees ha⁻¹), grafted on M.9 rootstock and trained as slender spindles. Four commercial product were applied: 1) Brevis, containing 150 g kg⁻¹ metamitron, 2) Ormorok, containing 84 g L⁻¹ NAA 3) Globaryl 100, containing 100 g L⁻¹ 6-benzyladenine and 4) Coupon, containing 0,48 ml L⁻¹ ethephon.

Chemicals were applied as foliar spray at a volume of 1000 L ha⁻¹. The experiment was carried out as randomized design with five replications of one tree each. Pest control and other orchard management practices were the same for all the trees in the study. Regular commercial treatment was applied when average fruit diameter was 10 mm and contained BA 100 mgL⁻¹ + NAA 8,5 mgL⁻¹. Since commercial treatment did not expressed satisfactory thinning effect, the following new thinning protocols were applied 28 days after full bloom when average fruit diameter was 19 mm:

Metamitron 250 mgL⁻¹

1. Metamitron 250 mgL⁻¹ + BA 150 mgL⁻¹
2. Metamitron 250 mgL⁻¹ + Ethephon 300 mgL⁻¹
3. BA 150 mgL⁻¹ + NAA 15 mgL⁻¹ + Ethephon 300 mgL⁻¹
4. Ethephon 300 mgL⁻¹ + NAA 15 mgL⁻¹
5. Ethephon 300 mgL⁻¹

Commercial treatment served as a control treatment. Spraying was carried out with a hand sprayer. No surfactant was used.

The effect on the yield, fruit quality and return bloom were recorded and evaluated according to the following measurements: (1) the yield per tree and per hectare, (2) number of fruit per tree; (3) fruit weight (g); (4) fruit diameter (mm); (5) fruit firmness (kg cm⁻²); (6) soluble solids (%); (7) iodine-starch index (scale 1-5); (8) total acid content (%); (9) number of flower buds per tree for next season (return blooming). At harvest fruit were counted and samples of 10 fruits per repetition were taken. All data were elaborated by analysis of variance, followed by means separation using LSD test at $p < 0.05$ level of significance.

RESULTS AND DISCUSSION

Fruit set of 'Gala' in 2021 was extremely high on unthinned trees. The commercial technique of BA and NAA at 10 mm fruit diameter was used to chemically thin fruitlets. The thinning effect of BA and NAA was low and hand thinning was needed to be done in order to obtain high quality fruits. As often happens when using NAA, fruit size was less than expected by the fruit number reduction (Dorigoni and Lezzer, 2007). As Basak (2006) reported that 'Gala' needs to be intensively thinned because it tends to produce a large number of small fruits and tends to bear biennially. Robinson (2006) reported the effect of

NAA + BA on twelve apple cultivars, noting that for 'Gala' the treatment did not develop pygmy fruit, on the contrary Cortens and Cline (2015) reported many pygmy fruit developed in Imperial Gala.

Table 1. The influence of delayed thinning on fruit mass and fruit diameter of 'Gala' apple

Treatment	Fruit weight (g)	Fruit diameter (mm)
Control	97±3,28bc	60,8±0,64cde
MM 250	90±3,95c	59,1±0,98e
MM250 + BA150	94±3,64c	60,1±0,72de
MM250 + E300	106±3,94ab	62,0±0,89bcd
BA150 + NAA15 + E300	111±2,32a	64,2±0,76a
NAA15 + E300	109±3,12a	62,8±0,71ab
BA150 + E300	104±1,97ab	62,4±0,52abc
E300	104±2,57ab	62,2±0,74abcd
Significance	***	***

* Mean separation within column by LSD test, $P = 0.05$

The weather condition were sunny and warm when treatments were applied. Regarding the chemical thinning results obtained in this experiment, significant thinning response occurred when tank mixed treatment of MM250 + E300, BA150 + NAA15 + E300 and NAA15 + E300 were performed. The mean fruit weight was enhanced only by the application of BA150 + NAA15 + E300 and NAA15 + E300 (table 1), and fruits that were larger compared to the control treatment was only when application of

Table 2. The influence of delayed thinning on chemical compounds, fruit firmness and return blooming of 'Gala' apple

Treatment	Iodine starch index (0-5)	Soluble solids content (%)	Total acids (%)	Fruit firmness (kg/cm ²)	Number of flower buds
Control	1,88±0,12b	11,2±0,11	0,27±0,01	10,2±0,11bc	52,0±15,03bc
MM 250	1,94±0,16b	11,6±0,14	0,31±0,02	10,7±0,28ab	17,2±1,56c
MM250 + BA150	1,72±0,08b	11,6±0,13	0,29±0,01	10,9±0,25a	50,4±16,48bc
MM250 + E300	1,74±0,10b	11,7±0,20	0,28±0,03	9,9±0,13cd	91,2±14,34a
BA150 + NAA15 + E300	2,06±0,16ab	11,7±0,20	0,29±0,01	9,9±0,31cd	112,4±9,40a
NAA15 + E300	1,96±0,16b	11,4±0,07	0,29±0,01	10,0±0,27cd	53,0±10,97b
BA150 + E300	2,04±0,15ab	11,4±0,08	0,29±0,01	9,6±0,10de	53,0±10,97b
E300	2,38±0,14a	11,2±0,21	0,27±0,02	9,1±0,17e	50,6±11,73bc
Significance	*	ns	ns	***	***

* Mean separation within column by LSD test, $P = 0.05$

treatment BA150 + NAA15 + E300 was performed, but the total yield (kg/per tree) was almost equal (not significantly different) to the control treatment (Figure 1). All other treatments (1, 2, 3, 7 and 8) did not thin the fruits significantly since the final fruit number was just slightly lower compared to the control treatment (Figure 1). It has to be taken into account that 'Gala' is considered as a hard to thin cultivar, which is probably the reason for such insignificant thinning (Stopar, 2007) for those treatments. For unknown reasons, when fruits are larger than 20 mm they are less susceptible to chemical thinners. It may be that with increasing size, the fruits become a priority sink over shoots (Robinson and Lakso, 2011). Ethephon was more effective when applied at fruit diameter of 18 mm than at 9 mm, and crop load decreased nonlinearly with increasing ethephon concentration (Marini, 1996). Jones and Koen (1985) reported that ethephon application resulted in erratic thinning response and is highly temperature dependent – high ambient temperatures especially in the days following application resulted in excessive thinning, however Yuan (2007) reported that ethephon thinned 'Golden Delicious' apples when applied at 20 mm stage of fruit development, and fruit thinning with ethephon was not influenced by increasing day/night temperatures. Ethephon sometimes also negatively affects fruit shape in some cultivars resulting in flat fruit (Basak, 2006).

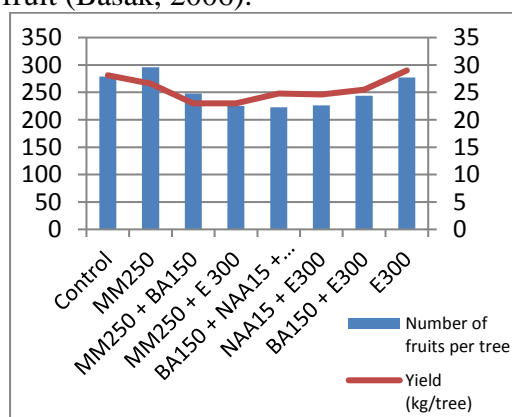


Figure 1. The influence of delayed thinning on productivity of 'Gala' apple

Considering return blooming, only MM250 + E300 and BA150 + NAA15 + E300 had an effect on the formation of flower buds for the next season. The least amount of the flower buds formed when MM250 was applied alone. The measurements of inner fruit quality parameters at harvest showed that ethephon increased fruit ripening and resulted in less flesh firmness of fruits compared to the control treatment (table 2).



Picture 1. Stage of fruitlets when treatments were applied

Only the application of MM250 + BA150 did increase fruit firmness (significantly different). Greene (2014) also reported the same results. From the results of our experiment it was seen that differences in % of soluble solids and total acid content were similar, but iodine starch index showed that only E300 treatment applied alone had a significant difference and resulted in more ripend fruits (table 2). None of the other treatments differed heavily from the control treatment.

CONCLUSIONS

Based on the results of this study, ethephon in combinations with other applied thinning chemical compounds could be used as 'last chance' agent in delayed apple fruit thinning 'Gala' variety.

Including all examined parameters, treatments with best results were MM250 + E300, BA150 + NAA15 + E300 and NAA15 + E300.

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ACCUMULATION OF HEAVY METALS IN ORGANS OF TALL FESCUE (*FESTUCA ARUNDINACEA* SCHREB.)

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Abstract

The tall fescue (*Festuca arundinacea* Schreb.) was sown on the flotation tailing dump of lead, copper and zinc mine. The aim of the study was to examine the possibility of phytostabilization of this substrate. Experiment was set up in central Serbia in april 2021. The one half of experiment plots was fertilized with organic and mineral NPK fertilizer, and the other half of plots were control plots without fertilizers. Flotation tailings have very unfavorable physical and chemical properties with increased content of heavy metals and low nutrient content. The content of all tested heavy metals (Cu, Cd, Ni, Pb, Zn, Fe), except manganese (Mn), in the tailings was above the maximum allowed quantities for arable land. The accumulated content of heavy metals in roots of tall fescue was in concentration that are toxic to most plant species. The most accumulated element in root and shoot was Fe. The concentration of heavy metals was higher in root than in shoots of tall fescue, and the calculated translocation coefficient was less than 1 for all tested heavy metals, except iron in fertilized plants. Compared to the control ones, plants in NPK treatments adopted more Cu, Cd, Pb, Ni, and less Fe, Zn and Mn. The tall fescue has accumulated most of the adopted heavy metals at the root, and we can classify it as an excluder plant for most tested elements.

Key words: *Festuca arundinacea*, flotation tailings, heavy metals, fertilization

INTRODUCTION

The harmful impact of mining activities is reflected in the disposal of large amounts of tailings on the surrounding soil (Lakić et al., 2016). Tailings are anthropogenic substrate with increased content of heavy metals and unfavorable characteristics. The growth of plants and mineralization on such substrates are limited due to lack of nutrients (Malić & Matko Stamenković, 2019). Therefore, recovery of such lands is necessary, and one of the best methods for that purpose is phytoremediation. Phytoremediation involves sowing of plants that can grow on unfavorable substrates and reduce content of polutans by their accumulation and transport (Pusz et al., 2021). Grasses (*Poaceae*) are good candidates for phytoremediation thanks to their rapid growth and the large biomass they form. One of the plant species from family *Poaceae* that can be used for this purposes is tall fescue (*Festuca arundinacea*), because of its ability

to grow on substrates with increased content of lead, copper, zinc, nickel and cadmium and high heavy metal remediation capacity (Li et al., 2021).

The aim of the research was to examine the growth potential of tall fescue at the flotation tailings dump and the impact of fertilization on the accumulation of heavy metals in the root and shoot.

MATERIAL AND METHODS

The experiment was set up on April 1st, 2021. on the slope of the flotation tailings dump of mine of lead (Pb), copper (Cu) and zinc (Zn) according to completely randomized block system with three replications. Before sowing, one half of the experimental plots were fertilized with combination of mineral NPK fertilizer (formulation 20:20:20) and organic NPK fertilizer (formulation 4:4:4) in amounts of 100 kg ha⁻¹ of mineral NPK fertilizer and 700 kg ha⁻¹ of organic fertilizer. The other half of plots were control plots without fertilization.

Sowing was performed on isohypses with row spacing of 50 cm. For experiment was used cultivar of tall fescue Kora from Institute for Forage Crops in Kruševac. The area of each individual plot was 10 m². The sampling of plants for laboratory tests was performed in October 2021. The concentrations of all metals were calculated according to standard curves obtained on the basis of the absorbances of a

series of standard solutions of known concentration. The data are calculated as the arithmetical means of three replications of each treatment, with the standard deviation (SD).

The microclimatic conditions on experimental field for period from sowing to sampling are shown in Table 1 (Road Weather Information System, 2022).

Table 1. Microclimatic conditions during the experimental period (April-October, 2021.)

2021.	April	May	June	July	August	September	October
Temperature (°C)	6.35	14.2	19.7	23.6	17.8	16.2	9.58
Precipitation (mm/m ²)	26.9	120.5	63.6	121.7	58.4	41.9	11.2
Temperature average = 15.3°C				Total precipitation = 444.2 mm/m ²			

The total amount of precipitation during this period was 444.2 mm/m². The highest content of rainfall was recorded in July in amounts of 121.7 mm/m². The average air temperature in experimental period was 15.9°C. The chemical reaction of substrate was neutral with very small differences between active and

substitutional acidity. This is a consequence of reduced content of mineral and organic acids in substrate (Andrejić, 2020). The flotation tailing is characterized by a reduced amount of nutrients (NPK) and increased content of heavy metals (table 2).

Table 2. The chemical properties of substrate (Andrejić et al., 2022)

Parameter	Fertilized	Control
pH in H ₂ O	7.02	6.85
pH in KCl	6.85	6.59
N (%)	0.002 ± 0,0001	0.005 ± 0,001
P ₂ O ₅ (mg/100 g soil)	0.93 ± 0,0004	1.08 ± 0,006
K ₂ O (mg/100 g soil)	10.30 ± 0,86	8.49 ± 0,04
Organic C (%)	2.73 ± 0,80	3.71 ± 0,25
Pb (mg/kg)	665.4 ± 21,9	655.3 ± 0,86
Zn (mg/kg)	1112 ± 33,9	1217 ± 49,3
Ni (mg/kg)	203.6 ± 3,26	217.2 ± 8,47
Cu (mg/kg)	308.5 ± 5,07	443.4 ± 2,30
Cd (mg/kg)	6.40 ± 0,03	7.33 ± 0,63
Mn (mg/kg)	953.9 ± 29,4	884.6 ± 50,9

High levels of carbon have been recorded in the tailings due to the presence of the organic compound xanthate added during ore extraction.

RESULTS AND DISCUSSION

The content of heavy metals in plant material is shown in table 3. The most accumulated element in root and shoot of tall fescue was

iron (Fe). Fe was accumulated in concentrations of 7.74 g kg⁻¹ in the root, and 3.59 g kg⁻¹ in shoot of control plants compared to 6.36 g kg⁻¹ in the root and 6.80 g kg⁻¹ in leaf of fertilized plants. In fertilized plants, a slight decrease in the amount of iron in root was observed, but its concentration in the shoot increased by almost 50%. The iron content in

the shoot of fertilized plants varied significantly with $SD \pm 2.58 \text{ g kg}^{-1}$. Significantly higher content of copper and nickel was observed in fertilized plants compared to non-fertilized ones in both organs, root and shoot. The largest amount of this two elements was accumulated in root of tall fescue, and much smaller part was transported to shoot. Fertilization affected the increased uptake of lead and cadmium. The accumulated content of these elements was higher in fertilized plants, but there were no significant differences compared to non-fertilized ones. A slightly larger amount of cadmium was

accumulated in roots (4.68 mg kg^{-1} in control plants and 5.08 mg kg^{-1} in fertilized ones) compared to shoots (3.29 mg kg^{-1} in control and 3.89 mg kg^{-1} in fertilized plants). In fertilized plants was recorded lower content of Zn and Mn compared to control ones. Lower content of these elements was detected in roots and shoots of fertilized plants compared to non-fertilized ones. Largest amount of both elements was accumulated in roots. In case of zinc, a significant variation in the accumulated amount was observed in shoot of plants with $SD \pm 96.6 \text{ mg kg}^{-1}$ in control plants and $SD \pm 131 \text{ mg kg}^{-1}$ in shoot of fertilized ones.

Table 3. Content of heavy metals in plant material

Metals (mg kg^{-1})	Control plants		Fertilized plants	
	Root	Shoot	Root	Shoot
Cu	53.04 ± 1.76	22.28 ± 0.36	77.3 ± 8.42	37.42 ± 4.40
Cd	4.68 ± 0.18	3.29 ± 0.39	5.08 ± 0.19	3.89 ± 0.16
Fe	7742 ± 576	3594 ± 38.4	6361 ± 686	6801 ± 2578
Mn	1369 ± 25.4	930.3 ± 8.83	1265 ± 123	827.9 ± 28.2
Ni	113.6 ± 4.55	35.60 ± 1.82	191.1 ± 8.21	49.23 ± 4.97
Pb	226.7 ± 9.65	57.91 ± 2.33	231.3 ± 24.0	88.09 ± 18.7
Zn	626.2 ± 57.8	549.8 ± 96.6	596.4 ± 63.6	384.8 ± 131

Our results are in line with results of Steliga & Kluk (2020) who examined the possibility of heavy metal uptake in organs of tall fescue growth at the soil contaminated with Pb, Ni, Cu and petroleum hydrocarbons and concluded that tall fescue most of adopted heavy metals accumulate in roots and significantly smaller amount transport to shoots. Same authors concluded that tall fescue has possibility to grow on substrates with increased content of pollutants and accumulate them in certain amount in its organs. The translocation coefficient (TF) was calculated for all examined elements. TF was less than 1 for all elements with exception of iron in fertilized plants. Therefore we can conclude that the tall fescue is an excluder for most of tested heavy metals and can be used for phytoremediation purposes. This is in accordance with results of Steliga & Kluk (2020) who concluded that the

use of tall fescue in phytoremediation achieves satisfactory results.

CONCLUSIONS

The flotation tailings are anthropogenic substrate with very unfavourable chemical characteristics. Such substrates are characterized by very low nutrient content and increased content of heavy metals. Tall fescue has ability to grow on such substrates and to accumulate certain amount of heavy metals in its root and shoot. The most of adopted content of heavy metals were accumulated in root of plants and the translocation coefficient was less than 1 for all tested heavy metals (Cu, Cd, Ni, Pb, Zn, Mn), except Fe in fertilized plants. Therefore tall fescue can be classified as an excluder for most tested elements and good candidate for use in phytoremediation purposes. Fertilized plants adopted higher content of Cu, Cd, Pb and Ni, and lower

content of Fe, Zn, Mn compared to control plants.

The high content of adopted heavy metals in the plant material is a consequence of their increased concentration in substrate.

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INFLUENCE OF AGROECOLOGICAL CONDITIONS ON PINOT NOIR AND PROKUPAC GRAPES AND WINES QUALITY

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Abstract

Aim of this research was to determine influence of locality agroecological condition on grapes and wine quality of Pinot Noir and Prokupac variety. The experimental vineyard was planted in 2005, with a planting distance between rows of 3 and with 1 m row space. The influence of air temperature on grape quality indicators was monitored, from veraison to the full maturity. To determine harvest moment samples was harvested on interval of 5 days. In paper were presents results of total acids (g/l), sugar content (%), pH values in grape juice-must, total phenols (mg GEA/l), and anthocyanins content (mg/g) in berry skin. For wine were present alcohol (vol. %), extract (g/l) and total phenolic content (mg GEA/l), also were present results of color intensity, color nuance and total acids (g/l). According to the obtained results, Pinot Noir variety had better results for total acids (6.37 g/l) and sugar content (22.0%) in grape juice compared to the other tested variety Prokupac (5.85 g /l; 20.3%). The pH value for Prokupac was lower (3.19) compared to Pinot Noir (3.27). The produced wine from Pinot Noir variety had similar results of alcohol content, extract, nuance and color intensity (12.5 vol.%; 22.01 g/l; 0.59; 0.30) as the wine of the Prokupac variety (12.2 vol.%; 21.08 g/l; 0.57; 0.40). A difference was found for total phenolic content in wine for Pinot Noir variety (1450.0 mg GAE/l), compared to the Prokupac wine, which had 1000.0 mg GAE/l. The results of the wine analysis indicate that rose wines were made from examined wine varieties in agroecological conditions.

Key words: *grapevine, locality, air temperature, quality of grapes and wine*

INTRODUCTION

The grapevine (*Vitis vinifera* L.) is one of the oldest cultivated plants and is considered to originate from the region between the Mediterranean basin and the Caspian Sea. It is believed that the first vine in today's Serbia was planted by the Roman emperor Probus in the 3rd century on the slopes of Alma Mons (Fruška gora) in the vicinity of Sirmium (Sremska Mitrovica), so the beginning of viticulture in Serbia, is connected to his name. The history of Serbian viticulture is longer than 1000 years - from the beginning of the Serbian state in the 8th and 9th centuries, and especially during the rule of the Nemanjić dynasty, from the 11th to the 14th century.

The territory of Serbia is characterized by very favorable agro-ecological conditions necessary for growing vines. The vineyards are mainly distributed at altitudes of 80 to 500 m. Temperature conditions are a limiting factor for growth. Meteorological factors besides other factors (variety, viticultural practices, soil, etc.) are essential for the manifestation of

the qualitative properties of grapes and produced wine (Ranković-Vasić et al., 2015a). Air temperature is a key driver of grapevine phenology and a significant environmental factor affecting yield and quality for a grapevine growing region. In cool and temperate regions, high quality vintages were associated with warmer than normal growing seasons for Pinot Noir (Skahill et al., 2022). The importance of climate for the production of high quality 'Pinot Noir' grapes and wine is well established. Generally speaking, 'Pinot Noir' makes uninteresting wines in hot climates, where the wines lack color. This suggests that climate change may affect wine quality in many 'Pinot Noir' producing wine regions (Duley, 2021). Pinot Noir is one of the most planted grape varieties in the world, occupying the 12th position in the world (10th place among wine-producing varieties) and occupying about 112 ha of planted area (Maia et al., 2019). Now 'Pinot Noir' has become an international variety and is grown in a large number of wine-growing regions around the

world. Its oenological potential is reflected in the interaction with different "terroir" conditions that result in specific elements of the sensory and quality characteristics of the wine (Ranković-Vasić, et al., 2015b).

Prokupac is the dominant grape variety in southern Serbia and it is used to make quality wines with a recognizable and unique taste. It is an autochthonous grape variety that produces table and quality red wines, and belongs to the ecological geographical group *Convar pontica*, *Convarietas balcanica*. In the last decade, the Prokupac variety has once again become the dominant grape variety in the wine regions of southern Serbia. Quality wines with a recognizable and unique taste are characteristic of this part of Serbia (Ćirković, et al., 2019). According to the data of the Republic Institute of Statistics in 2018, the production of grapes in Serbia amounted to about 150,000 tons, with a clear trend of increasing the production of autochthonous grape varieties, especially Prokupca, as one of the most popular autochthonous grape varieties (Milanović et al., 2021).

The fruit of the vine in the narrower sense is a berry characterized by a complex biochemical composition. It contains water, sugars, amino acids, organic acids, vitamins, minerals, microelements, etc. Polyphenolic compounds are also formed in the berry, which gives it a specific taste, smell, and color, which are extracted into wine. The grape as a collective fruit of berries is the only fruit in the composition in which a larger amount of tartaric acid is present (Riaz et al., 2002). The anthocyanin content in grape berry skins is an important factor determining wine quality (Mori et al., 2005).

This research aimed to determine the influence of meteorological characteristics on the quality of grapes and wines wine varieties Pinot noir and Prokupac, which are located in the wine-growing area of Grocka in the Belgrade region.

MATERIALS AND METHODS

Experiment was set up in "Radmilovac", which is located in Grocka wine growing area in the Belgrade region. The geographical position of the building is 44° 45' 24.66'' north latitude

and 20° 34' 54.50'' east longitude. The altitude of the site is 153 m with a slope of 3%. The experimental plantation was built in 2005, with a planting distance between rows of 3 x 1 m, and the tested varieties were grafted on a vine rootstock Kober 5BB (*Berlandieri x Riparia*). Two cultivars were examined in this paper Pinot Noir and Prokupac.

Synonyms for the variety Pinot Noir are: 'Pinot Noir' (France), 'Blau Burgunder' (Austria), 'Pignola' (Italy). The variety is widespread in the area of Austria, Germany, Slovakia, Switzerland, Hungary, Romania, Croatia, and Montenegro. In Serbia, it is grown in almost all vineyards. The vine is moderately lush. The flower is morphological and functionally hermaphroditic. The cluster is small, compact, and cylindrical, with a short stalk. The berry is small, round, juicy, and dark blue. It matures at the end of the 1st epoch. Yield: 6.000-12.000 kg/ha (Žunić and Garić, 2017). The Pinot Noir variety is intended for the production of high-quality and top-quality red wines.

Synonyms for the Prokupac variety are: 'Crnika', 'Nisevka', 'Rskavac', 'Nikodimka' (Serbia), 'Zarčin' (Bulgaria), 'Skopsko crno' (Macedonia), 'Majski čorni' (Russia). It is an autochthonous variety of Serbia. It is grown in Macedonia, to a lesser extent in Bulgaria, and sporadically in Russia. In Serbia, it is grown in the central part, in Kosovo and Metohija, and some places in Vojvodina. The vine is very lush. The flower is morphological and functionally hermaphroditic. The cluster is medium-sized, cylindrically conical, moderately compact. The petiole of the bunch is short and herbaceous. The grapes ripen between 3 and 4 epochs. Yield: 15.000 – 20.000 kg/ha. The Prokupac variety is intended for the production of high-quality and top-quality red wines. As a result of many years of work on the clonal selection of indigenous varieties, eight clones of the variety Prokupac have been recognized, and another 15 clones are in the process of recognition (Marković et al., 2007a, b).

The influence of air temperature on grape quality indicators was monitored, from veraison to the full maturity. Data logger was placed within the bunch zone and also used in

the experimental vineyard. To determine harvest moment samples were harvested on intervals of 5 days.

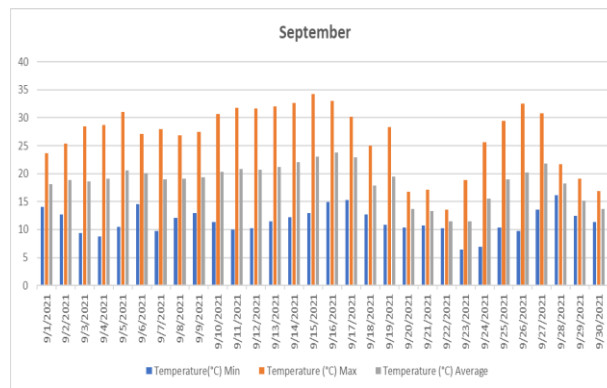
Grape quality parameters were analyzed: sugar content (%), total acids (g/l), pH in grape juice-must, total phenols (mg GEA/l), and total anthocyanins content (mg/g) in berry skin. Sugar content of the must (%) was established by refractometer (Pocket Atago Pal 1) and total acids content in the must was determined by titration with n/4 NaOH. The pH of the grape juice was determined using a pH meter.

Total phenol content in berry skin was determined by the spectrophotometric method (AOAC, 1984). Total anthocyanin content was determined by the spectrophotometric method (AWRI, 2009) in berry skin. Grape processing was done within the microvinification process. 10 kg of grapes was processed and for alcoholic fermentation was used yeast Lalemand (Kanada).

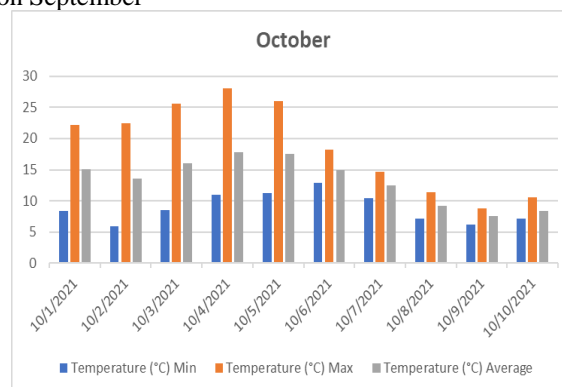
The wines are not clear or filtered. Chemical analysis of wine was done after 6 months and included one wine per variety. Official methods published in the ESS regulation were used for standard wine analyses (ECC - Commission Regulation No 2676/90 concerning the establishment of common analytical methods in the sector of wine, 1990). Wine quality parameters that were analyzed: alcohol (vol. %), extract (g/l), total phenolic content (mg GEA/l), color intensity, color nuance and total acids (g/l).

RESULTS AND DISCUSSIONS

Meteorological characteristics have a significant impact on ripening dynamics and quality of grapes. The content and concentration of phenolic substances, sugars and total acids in the berry, apart from the variety and the applied viticultural practice, also depend on the temperature conditions during the ripening period (Ranković-Vasić, et al. 2022).

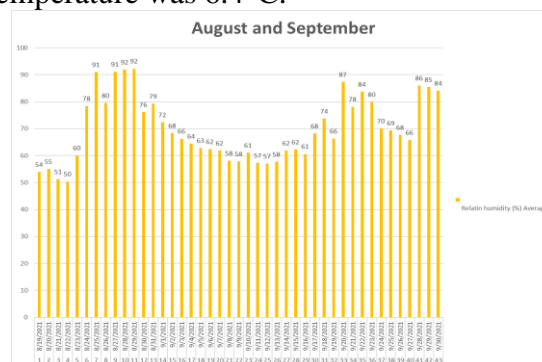


Graph. 1 Display of min, max, and average temperature on September

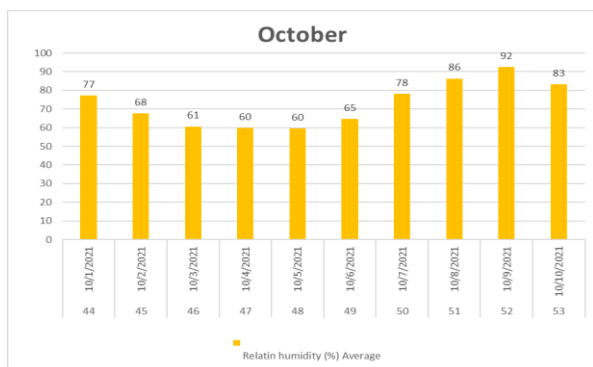


Graph 2. Display of min, max, and average temperature on October

Graphs 1 and 2 show temperatures (min, max, and average) from the veraison period to the period of full maturity. Based on the temperature values, a correlation can be determined between the temperature and the chemical composition of the grapes. The highest recorded temperature during the examined period was 34.2°C, while the lowest temperature was 6.4°C.

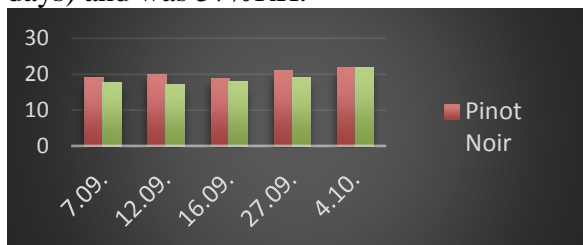


Graph. 3. Humidity display during August and September



Graph. 4. Humidity display for October

Graphs 3 and 4 show the oscillations of air humidity from the period of veraison to the period of full maturity. The highest value of relative air humidity was recorded at 20.9. and was 87%RH, while the lowest value was recorded at 11.9. and 12.9. (two consecutive days) and was 57%RH.

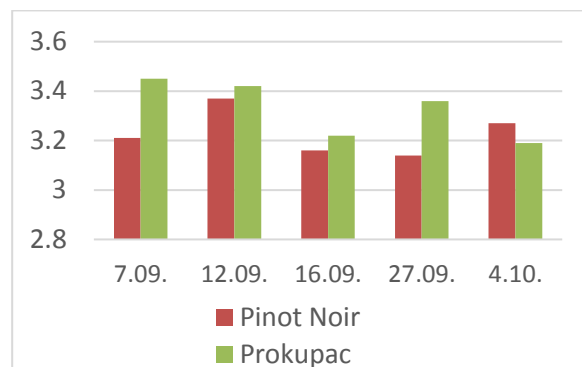


Graph. 5. The value of sugar in berries

The amount and the distribution of precipitation could affect the quality of grapes and wines. Graph 5 shows the values of sugar in the berry. The concentration of sugar in the berry increases with the ripening of the berry. For the variety Pinot Noir the highest recorded value of sugar was 22% and the lowest value was 17.2%. While for the variety Prokupac, the highest value was 21.8%, and the lowest was 17.2%.

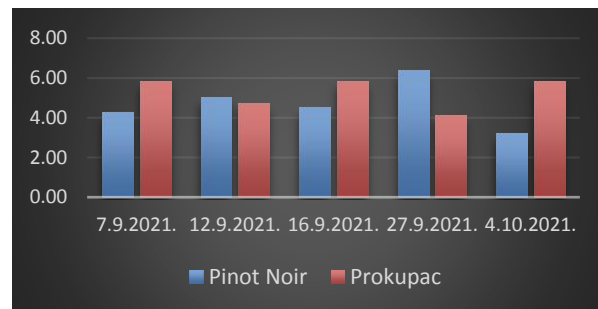
Table 1. Results of the analysis of the chemical composition of wine of the tested varieties

	Prokupac	Pinot Noir
Alcohol (vol. %)	12.2	12.5
Extract (g/l)	21.08	22.01



Graph. 6. pH value in grape juice

Graph 6. pH values in grape juice are shown. The highest value of the variety Pinot Noir was 3.37, while for the variety Prokupac it was 3.45. It can also be seen from the graphs that the lowest pH value of the cultivar Pinot noir was 3.14, and of the cultivar Prokupac 3.19.



Graph. 7. The value of total acids in grape juice

In the period 27.9. the variety Pinot Noir recorded the highest value for the values of total acids in grape juice (6.37 g/l), while the variety Prokupac recorded similar values (5.85 g/l) for the period 4.10.

Color intensity	0.40	0.30
Color nuance	0.57	0.59
Total phenolic content (mg GEA/l)	1450	1000

Phenolic compounds have a significant impact on the quality of red wines, primarily by giving them the appropriate character, color, and taste, and the positive effect of phenolic compounds from wine on human health has

been. The content of total phenolic compounds in wines, in addition to the variety, clone, also depends on the ecological factors of the locality, the maturity of the grapes, the duration of maceration, and other procedures during wine production (Radovanović et al, 2010; Pržić i Marković, 2019; Petrović et al., 2019).

From table 1 it can be seen that the variety Prokupac has a higher color intensity (0.4) and the content of total phenols (1450 mg GEA/l) compared to the variety Pinot Noir (0.3; 1000 mg GEA/l). The produced wine from Pinot Noir variety had similar results of alcohol content and extract (12.5 vol.%; 22.01 g/l) as the wine of the Prokupac variety (12.2 vol.%; 21.08 g/l).

CONCLUSIONS

Meteorological characteristics of locality have a significant influence on ripening dynamics and quality of grapes. The content and concentration of phenolic substances, sugars and total acids in the berry, apart from the variety and the applied viticultural practice, also depend on the temperature conditions during the ripening period. An increase in the air temperature could produce an advance in the start of phenophases and an effect on the ripening length in grapes. The results of the wine analysis indicate that rose wines were made from examined wine varieties in agroecological conditions of Grocka wine growing region were in category of good quality wines.

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Plant Growth-Promoting Rhizobacteria: Their Potential Use in Field Crops and Importance in terms of Agricultural Sustainability

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Abstract

The natural balance of the Climate of Earth has deteriorated due to many human-driven factors such as population growth, industrialization, misuse of agricultural lands and the destruction of natural resources, and the possibility of the fear of a global crisis, which has had adverse effects on many fields up to now, especially in agriculture, has become a matter of concern. The climate crisis substantially affects agricultural production and causes major losses in agricultural productivity, since agricultural production is dependent on the direct use of natural resources. Therefore, the possibility of the fear of a global food shortage makes the issue of sustainability in agriculture more important. In order to minimize the use of chemical fertilizers and pesticides for sustainable agriculture, alternative choices that are harmless both to the environment and nature such as rhizobacteria are preferred. These beneficial microorganisms, called plant growth-promoting rhizobacteria, PGPR; in consequence of their use as biofertilizers, it is of prime importance with regards to their contribution to the continuity of agricultural sustainability and lower cost compared to chemical fertilizers. In addition to their plant growth-promoting effects, PGPRs also stand out with their use in biological warfare. In this review, the use of plant growth-promoting bacteria in field crops and their importance for the continuity of agricultural sustainability were examined.

Key words: plant growth promoting rhizobacteria (PGPR); biofertilizers; biocontrol.

INTRODUCTION

The use of chemical fertilizers and pesticides is common in agricultural activities to increase agricultural productivity and efficiency. However, it is known that the use of chemicals in agricultural activities causes significant agricultural losses by leading to negative effects such as salinity and nutrient deficiency in agricultural soils in the long term. In order to prevent this agricultural loss and to ensure agricultural sustainability, it is necessary to

reduce the use of chemicals and to move in to alternative methods that can be used instead.

Today, it is aimed to reduce greenhouse gas emissions in order to tackle the effects of climate crisis and global change and to minimize their effects. 10-15% of greenhouse gas emissions in the World are caused by agricultural activities (FAO, 2021), and chemical fertilization, which is considered to be a part of these agricultural activities, has a crucial place among the activities that cause greenhouse gas emissions.

Therefore, the possibilities of using beneficial microorganisms such as rhizobacteria that are harmless to nature and the environment and that encourage plant production have been studied. The aim of the study; for a sustainable agriculture, is to study the possibilities of using harmless practices that can increase the crop yield and quality of the plant while reducing the use of chemical inputs. The possibilities of struggle using these microorganisms against stress conditions that adversely affect plant growth, such as drought, which are come upon as a result of climate change, were researched at the same time. This study was carried out in order to determine the potential of innovative and environmentalist solutions that can adapt to the changing world conditions in agriculture.

Recently, reducing the use of chemical fertilizers in agricultural production has become an important issue in terms of climate crisis and agricultural sustainability. In order to minimize the negative effects of chemical fertilizer use, alternative fertilization methods based on the use of organic or biological fertilizers are being developed. Biofertilizers, which are a sustainable and environmentally friendly form of fertilization methods, are produced from live microorganisms. The purpose of using biofertilizer; reducing the use of chemicals in agriculture and ensuring agricultural sustainability, promoting plant growth without harming the environment and natural resources, while increasing quality. The most important group of biofertilizers is root bacteria that increase plant growth.

Rhizobacteria, which are free-living in the roots of plants, promoting plant growth, are used in biological control and as biological fertilizers, are referred to as plant growth-promoting rhizobacteria (Altın and Bora, 2005). Rhizobacteria, that is to say, plant growth-promoting bacteria, are called PGPR

(Plant Growth Promoting Rhizobacteria) briefly.

Rhizobacteria can be used as a biological fertilizer (BG) since they tie elements such as nitrogen and phosphorus in plants, they can also stand out with their use against plant pests and diseases. In addition to this, it has been predicted as a result of some researches that they can be used against situations that may adversely affect the growth and development of plants such as drought and salt stress.

In this review, the possibilities of use and the effect mechanism of plant growth-promoting bacteria practice in field crops grown in Turkey were researched, and its importance in terms of agricultural sustainability has been studied.

Effect Mechanisms of Rhizobacteria

Plant growth-promoting rhizobacteria have two effect mechanisms on plants: direct and indirect.

Directly:

- I. Fixation of free nitrogen that is in the air,
- II. Phosphate solubilization,
- III. Synthesis of plant hormones,
- IV. Dissolution of mineral substances,
- V. It substantiates the synthesis of enzymes that adjust hormone levels, by providing the plant with the compound produced by the bacteria or catalyzing the intake of nutrients from the environment

Indirectly:

- I. Limiting the production of iron, which is beneficial for the pathogen, with the siderophores they produce,
- II. Antibiotic production,
- III. Stimulation of systemic endurance,
- IV. Production of Antifungal metabolite,
- V. It substantiates so by competing with pathogens in terms of food and space (Çelik et al., 2020)

While through the practice of plant growth-promoting bacteria, the germination rate, the development of root, crop yield, leaf area, chlorophyll rate, nitrogen rate, protein rate, hydraulic activity, drought tolerance, root and stem weight increase, the aging of the leaves is delayed and resistance to some diseases is provided (Çakmakçı, 2005).

Plant Growth Promoting Rhizobacteria

Some species that belong to *Acetobacter*, *Acinetobacter*, *Achromobacter*, *Aereobacter*, *Agrobacterium*, *Alcaligenes*, *Artrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Clostridium*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Klebsiella*, *Micrococcus*, *Pseudomonas*, *Rhizobium*, *Serratia*, and *Xanthomonas* are used as plant growth promoters (Çakmakçı, 2005). *Rhizobium*, *Clostridium*, *Azotobacter*, *Klebsiella*, *Bacillus*, *Amylobacter* bacteria are known for their nitrogen-fixing features.

Studies on Their Use in Field Crops

The use of plant growth-promoting rhizobacteria in field crops and the studies related to their potential use were examined and the results were evaluated.

When the previous studies in the literature were examined, it was observed that the effect of plant growth promoting bacteria application on yield and quality parameters as a biological fertilizer, the possibilities of use in biological control against plant diseases and damages, and its role in reducing the negative effects of various stress factors on the plant were observed.

Use as Biological Fertilizer

Çakmakci et al. (2007) reported that some bacteria increased stem weight in wheat, the highest root and stem weight was obtained with *P. polymyxa* application, followed by *P. Putida* and *B. Megaterium*. Kadioğlu and Canbolat (2019) found that *P.agglomerans* and *A.agilis* bacteria were effective in their study

to determine the effects of plant growth promoting rhizobacteria (PGPR) on wheat and corn plant development in different environments. Soysal et al. (2020), it was reported that the highest yield was obtained from the application of *Bacillus atrophaeus* bacteria, in order to determine the effects on the development and yield of durum wheat variety.

Mutlu et al. (2019) applied microbial fertilizer containing 3 different bacteria (*Bacillus megaterium*, *Pantoea agglomerans*, *Pseudomonas fluorensens*) to the *Lolium perenne* plant and determined that high-dose microbial fertilizer application provided superior grass quality. Aşık and Arıoğlu (2019) reported that *Bradyrhizobium* sp. reported that the bacterium had a positive effect on the fatty acids in the plant. Demirbaş et al. (2020) reported that plant growth promoting bacteria application had significant effects on the yield and nutrient uptake of sugar beet plants. Simsek et al. (2021), in their study on the sugar beet plant, reported that the bacterial application had a significant effect on the yield increase in the plant. Dogan et al. (2007) reported that by inoculating the peanut plant with bacteria, nitrogen input can be reduced cheaply and nitrogen can be added to the soil, and the effects of environmental problems that may be caused by the use of chemical nitrogen can also be reduced. Tozlu et al. (2012), on the other hand, reported that bacteria promoting plant growth in *Phaseolus vulgaris* plant increased yield and growth in the plant. They also reported that PGPRs suppressed diseases caused by bacterial or fungal pathogen infections. Altunlu et al. (2019) reported that the application of microbial fertilizer containing plant growth promoting bacteria in sweet corn plant was effective on germination, plant growth, yield increase and fruit quality. Yilmaz et al. (2022), on the other hand, found

that PGPR and AMF combined gave positive results in plant protein content in their study on soybean plant.

Uysal (2018) reported that the bacteria applied on the early potato plant shortened the plant emergence time, increased the plant growth and increased the tuber yield.

Özyılmaz and Benlioğlu (2012) reported that the application of phosphate solvent *Burkholderia cepacia* and *P. fluorescens* bacteria increased the dry weight of the cotton plant and contributed significantly to phosphorus uptake, and also reported that it was effective against *Verticillium* wilt disease. Z. Taşkın and Bilgili (2020) reported that the application of nitrogen fertilizer and bacteria to hot climate grass plants together increased the color and quality of the grass, but the application of bacteria alone did not have a positive effect.

Use in Plants under Stress Conditions

It has been reported that metabolites synthesized by rhizobacteria under stress conditions in plants increase resistance to stress conditions in plants (Küçük and Almaca, 2020). It has been reported that the production of IAA, cytokinins, antioxidants, and ACC deaminase increase with the application of rhizobacteria in plants (İpek et al., 2017). In addition, they are known to be used with the effect of reducing the amount of ethylene, which is increased by the plant under stress conditions. While ethylene production increases in plants under stress conditions, ethylene synthesis inhibitors and regulatory substances decrease. Bacteria containing ACC deaminase may provide protection against the inhibitory effect of stress in plants by reducing the plant ethylene level (Çakmakçı, 2009; Samancıoğlu and Yıldırım, 2015). In addition, Samancıoğlu and Yıldırım (2015) emphasized the importance of bacterial applications that

promote plant growth against drought stress in their study.

Selem et al. (2021), it has been reported that *Bacillus megaterium* bacteria, known as P solvent, reached high values in various parameters and is suitable for arid conditions against different drought applications in *C.officinalis* plant. İnal et al. (2021) applied 3 different bacteria (*Pseudoalteromonas tetraodonis*, *Pseudomonas agarici*, *Brevibacillus choshinensis*) showing ACC deaminase effect on *Triticum aestivum* and reported that it reduced drought stress. Cirka et al. (2022), on the other hand, concluded that the application of rhizobacteria (*Bacillus megaterium*, *Azospirillum lipoferum*) under drought stress in *Vicia faba* plant is not very effective.

Pordel et al. (2019), it was reported that as a result of bacterial application against early aluminum stress in *Triticum aestivum*, *Pseudomonas fluorescens* bacteria reduced the negative effect of aluminum stress. Yolci et al. (2022) reported that the bacteria *Azospirillum lipoferum*, *Bacillus megaterium* and *Frateuria aurentia* were quite effective in tolerating boron toxicity in the medicinal sage plant compared to the control.

Tuncturk et al. (2021), on the other hand, found that *Azospirillum lipoferum* bacteria applied to the *Glycine max* plant under water stress conditions reduced the damage to the plant.

Çakmakçı and Karagöz (2020) reported that bacteria promoting plant growth in sugar beet plant positively affect plant growth, yield and nutrient uptake performance under stress conditions and alleviate water-induced stress.

CONCLUSION

When all the studies are evaluated, it can be said that the plant growth-promoting bacteria application positively affects the yield and quality of field crops. According to the results

of the research, better results were obtained from the use of chemical fertilizers and rhizobacteria when they were combined together. However, it cannot be said that a new application that can replace the use of chemical fertilizers and pesticides has been achieved in our country.

Determining the relationship between plant growth promoting rhizobacteria and the plant, determining the tolerance under stress conditions and the effect on yield and quality of the plant; It has a great importance in terms of adopting a sustainable agriculture approach compatible with the environment. Based on the literature research, it is seen that the resistance of plants to stress conditions increases with the application of PGPR and the plant growth and development are positively affected by its use as a biofertilizer. By increasing the use of these rhizobacteria, it can be aimed both to reduce the negative effects of chemical use and to alleviate the increasing costs of chemical fertilizers in today's conditions.

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CURRENT ECOSYSTEM MODELING APPROACHES

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Abstract

Ecosystem management is a complex management system that includes interacting organisms, processes and scientific disciplines. As single-species focused management systems have not been able to provide a through solution to applied ecology, ecosystem-based management systems have become the main focus in this area. Ecosystem-based management involves various actions to provide an understating of the ecosystem components, dependencies, interactions and feedback of the components. Along with building an ecosystem model, various software tools and websites are used to visualize and paint the desired picture of the ecosystem models to provide better understanding and point the missing components.

Recently, machine learning models are used to identify the solutions and provide overview for future scenarios in ecosystem modeling. This study provides an overview of the potential ecosystem models and their use in ecosystems with simple examples. Also, the main modelling tools that are used for various purposes in forest and marine ecosystems. Tools to be explained mainly focus on commonly used and relatively new forestry modeling tools.

Specifically, we provide an insight to terrain and vegetation modeling tools and their capacity. Managing an ecosystem is a challenging process as there is a limited data and balancing ecosystem components might not meet the model goals.

In this case, modeling involves certain uncertainties. This study also provides main types of uncertainties and certain strategies to address these challenges.

Many modeling tools and recent studies use machine learning in ecosystem modeling. As machine learning models can vary for different scientific fields, the end result has similar outcomes, solutions, challenges and failures. This study provides an overview of applications in ecosystem services, commonly used machine learning model groups in ecosystem services and the certain mistakes to avoid when creating a machine learning model.

Key words: ecology, ecosystem, modelling, machine learning

INTRODUCTION

“Ecosystem” refers to a dynamic complex of communities of plants, animals and microorganisms and their environments interacting as a functional unit. An ecosystem might include very few components or a circular system among predator, prey, vegetation etc. Main types of ecosystems are:

- Marine Ecosystem
- Coastal Ecosystem
- Freshwater Ecosystem
- Forest Ecosystem
- Dryland Ecosystem
- Island Ecosystem
- Mountain Ecosystem
- Polar Ecosystem
- Cultured Field Ecosystem

“Ecosystem services” are defined as human benefits resulting from interactions between components of an ecosystem, including supply (food), regulation (flood control), cultural (recreation), and supportive services (nutrient cycling).

For years, single-species based approaches in ecosystem models have been used to demonstrate the relationship between the species and their environment.

These kinds of models had shown a limited perspective to look at the species and environment relationship.

There are many parameters that affect the species, environment and the relationship. When an ecosystem model excludes these parameters, the accuracy of the model can decline and will not be able to illustrate the real relationship and interactions. In recent years, scientists have started to use more complex ecosystem models that include interactions of all ecosystem components rather than using single-species focused models.

Ecosystem models can change and vary according to their components and the purpose of the model.

A model can demonstrate very few components with schematic representations of their interactions or can use mathematical equations to measure the interaction between components.

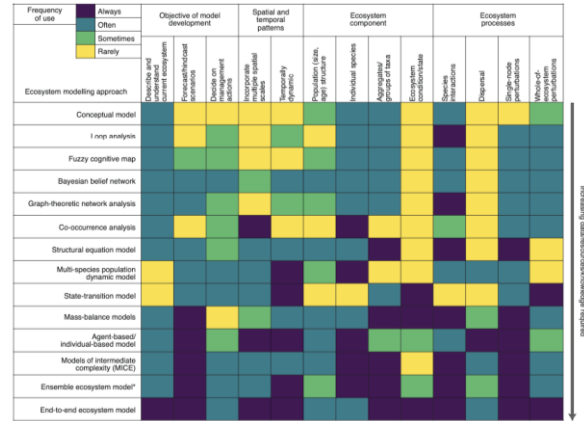


Figure 1: Common ecosystem modeling approaches (Geary et. al.)

Ecosystems can be measured and modeled according to their structure and components. There are many ways to apply an ecosystem modeling approach, and in Figure 1, Geary et. al. summarized the common ecosystem modeling approaches and their frequency where each approach is used for different purposes.

Creating an ecosystem model is a starting point to visualize and understand the ecosystem. Along with the modeling approaches, modeling tools are used to create visual representations, build future scenarios and detect issues related to ecosystems. Also, machine learning models are being used to create ecosystem models, show possible interactions, build recovery methods for the components and avoid possible extinction events in the future.

This study represents a literature review to explain the common ecosystem modeling approaches, ecosystem modeling tools (terrain and vegetation modeling), machine learning models that are used in ecosystem services and common mistakes in these machine learning models.

MATERIALS AND METHODS

In this study, literature about ecosystem modeling approaches, ecosystem modeling tools, machine learning in ecosystem services and uncertainty in ecosystem models’ subjects were reviewed and summarized.

Ecosystem Modeling Approaches

Ecosystems are unique organisms and the way it is modeled depends on the components in the system and the purpose of the model. Modeling an ecosystem usually starts with a schematic representation of the main components and their simple interactions. This approach is called conceptual interaction networks. In this method, components are drawn or named and the interaction between components are drawn manually.

Another approach is representing ecosystem interactions as a chain of probabilistic events that show how changes in the probability of one ecosystem component flow to affect the probability of another ecosystem component to change. This approach is called Bayesian networks. These patterns can be represented as a graphical structure or a chain of possibilities. Network theory, which is another common approach, encompasses a large collection of ecosystem modeling approaches based on simple parameterization of interaction networks. These can range from weighted networks to structural equation models.

Dynamic systems theory approach that focuses on change of ecosystems in a time period. These models are defined as more complex models and data requirement of the model is higher than other models.

These kinds of models usually are used to species re-introduction to an environment and their long-term effects, the consequences of invasive species in an ecosystem.

End-to-end ecosystem models are, however, widely used models in last decades.

These models include the steps of other models and create a complete model with visual interactions and equations. End-to-end models

are useful to predict component interaction and future of interactions and species.

Ecosystem Modeling Tools

When it comes to tools to model an ecosystem, there are many software and websites to visualize a model. Different tools are used in different ecosystems. For example, there tools that are specific to marine ecosystems and tools that focus on forest estimations. To give some examples to tools that are used in forest ecosystems, FORECAST is a deterministic stand-level forest model operating in annual time steps.

The stand growth and ecosystem dynamics projection is based on a representation of the rates of key ecological processes that regulate the availability and competition of light and nutrient resources.

LPJ Guess is a process-based dynamic vegetation-terrestrial ecosystem model designed for regional or global studies. Such models are commonly known as dynamic global vegetation models (DGVMs).

EFIMOD is a spatially open individual tree-based model that simulates biological transformation in tree-soil systems, recently enhanced by the addition of a root competition model.

A simulated stand consists of individual trees interacting with neighboring trees. Each tree creates a shade zone and a feeding zone, the size of which depends on the tree size.

The shape of the rooting zone of a single tree is defined by the availability of nitrogen in the soil and its interaction with neighboring trees.

Other powerful tools are used in spatializing ecosystem services according to the purpose of the study (Figure 2).

Tool used to spatialize ecosystem services	Description of tool
Integrated valuation of ecosystem services and tradeoffs – InVEST*	"InVEST is an open source software model used to map and value goods and services from nature in sustaining human life"
Soil and water assessment tool– SWAT**	It was developed as a conceptual and sustainable model to assist water resources managers in assessing water resources in basins.
Artificial intelligence for ecosystem services– ARIES**	ARIES provides an intelligent modeling platform that can create complex ecosystem services models.
Fog Prevention to improve stream flow in the tropics– FIESTA**	Model used for national scale fog suppression estimates and its potential impact on water resources, with emphasis on water production service. In addition, it evaluates the energy balance and horizontal precipitation.
Integrated multi-scale models of ecosystem services– MIMES	Spatialization and valuation model of ecosystem services
Co\$ting Nature**	Model of spatializing ecosystem services and prioritizing protected areas
Ecological Asset Inventory and Management– EcoAIM	A spatialization model of ecosystem services and stakeholder preference, a GIS optimization model that balances ecosystem service variables and risk analysis, including metric weighting of stakeholder preference
ECONOMETRIX	Calculates changes in ecological functions using a holistic focus that requires in situ collection (describing data) of the physical characteristics of an area
The Global Unified Metamodel of the Biosphere– GUMBO	Model used to simulate global dynamics and interactions with natural capital

Figure 2: Tools to spatialize ecosystem services

Along with spatializing tools, in forestry, there are tools that specifically focus on terrain or vegetation modeling. To give an example to these tools, TERRASOLID is a powerful terrain modeling software package, and it has TerraScan which is the main application to be used in terrain modeling. Terragen is another modeling tool that is able to produce very realistic images of landscapes with reasonable terrain, showing surface coloring, detailing, shadows, clouds, atmosphere, lighting, water and caustic effects. Object Raku Technology is another tool that is mainly used for accurate identification of timber types from LiDAR data.

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Machine Learning in Ecosystem Services

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Machine learning models are powerful way to create ecosystem models and these models require accurate information and data. In the study by Schowen et. al., a large number of models did not use enough data point to create an accurate model. 67% of the models did not report any hyperparameter tuning, 59% did not report the generalizability of the model, 43% failed to report anu model settings and 28% did not show the software used in the model.

Uncertainty in Ecosystem Models

Uncertainty is an essential component of managing complex systems. In ecosystem models, it can make uncertainty clearer by identifying key sources and their consequences.

Ecosystem models basically measure three characteristics:

1- Generality (Can the model be applied to various scenarios?)

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After completing an ecosystem model, the characteristics of the model can be checked in different phases of the model. Uncertainty can be in parameters, process, model/structure or future/scenarios. According to the study by Geary et. al., there are certain ways to detect the uncertainty in ecosystem models and also there are solutions to deal with the uncertainty.

RESULTS AND DISCUSSION

Ecosystem models are key factors to predict the future of environment, species, and natural processes. From single-species based approaches to more complex ecosystem-based models have been used more widely and this give the ecosystem services a dynamic to change and improve in time.

In a rapidly changing world, ecosystems are crucial to protect to provide a sustainable use of the planet. To be able to protect and predict, an accurate and determined model can be useful to understand the ecosystems and ecosystem services. In these models, data requirements can be difficult to obtain, for this reason, along with the models, data collection techniques in environmental sciences can be improved to provide more complex data for the models.

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models that are used in ecosystem services and common mistakes in these machine learning models.

MATERIALS AND METHODS

In this study, literature about ecosystem modeling approaches, ecosystem modeling tools, machine learning in ecosystem services and uncertainty in ecosystem models’ subjects were reviewed and summarized.

Ecosystem Modeling Approaches

Ecosystems are unique organisms and the way it is modeled depends on the components in the system and the purpose of the model. Modeling an ecosystem usually starts with a schematic representation of the main components and their simple interactions. This approach is called conceptual interaction networks. In this method, components are drawn or named and the interaction between components are drawn manually.

Another approach is representing ecosystem interactions as a chain of probabilistic events that show how changes in the probability of one ecosystem component flow to affect the probability of another ecosystem component to change. This approach is called Bayesian networks. These patterns can be represented as a graphical structure or a chain of possibilities. Network theory, which is another common approach, encompasses a large collection of ecosystem modeling approaches based on simple parameterization of interaction networks. These can range from weighted networks to structural equation models.

Dynamic systems theory approach that focuses on change of ecosystems in a time period. These models are defined as more complex models and data requirement of the model is higher than other models. These kinds of models usually are used to species re-introduction to an environment and their long-term effects, the consequences of invasive species in an ecosystem.

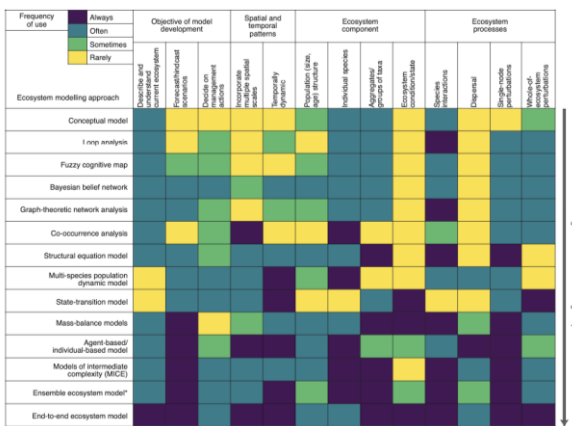


Figure 1: Common ecosystem modeling approaches (Geary et al.)

Ecosystems can be measured and modeled according to their structure and components. There are many ways to apply an ecosystem modeling approach, and in Figure 1, Geary et al. summarized the common ecosystem modeling approaches and their frequency where each approach is used for different purposes.

Creating an ecosystem model is a starting point to visualize and understand the ecosystem. Along with the modeling approaches, modeling tools are used to create visual representations, build future scenarios and detect issues related to ecosystems. Also, machine learning models are being used to create ecosystem models, show possible interactions, build recovery methods for the components and avoid possible extinction events in the future.

This study represents a literature review to explain the common ecosystem modeling approaches, ecosystem modeling tools (terrain and vegetation modeling), machine learning

End-to-end ecosystem models are, however, widely used models in last decades. These models include the steps of other models and create a complete model with visual interactions and equations. End-to-end models are useful to predict component interaction and future of interactions and species.

Ecosystem Modeling Tools

When it comes to tools to model an ecosystem, there are many software and websites to visualize a model. Different tools are used in different ecosystems. For example, there tools that are specific to marine ecosystems and tools that focus on forest estimations. To give some examples to tools that are used in forest ecosystems, FORECAST is a deterministic stand-level forest model operating in annual time steps. The stand growth and ecosystem dynamics projection is based on a representation of the rates of key ecological processes that regulate the availability and competition of light and nutrient resources.

LPJ Guess is a process-based dynamic vegetation-terrestrial ecosystem model designed for regional or global studies. Such models are commonly known as dynamic global vegetation models (DGVMs).

EFIMOD is a spatially open individual tree-based model that simulates biological transformation in tree-soil systems, recently enhanced by the addition of a root competition model. A simulated stand consists of individual trees interacting with neighboring trees. Each tree creates a shade zone and a feeding zone, the size of which depends on the tree size. The shape of the rooting zone of a single tree is defined by the availability of nitrogen in the soil and its interaction with neighboring trees.

Other powerful tools are used in spatializing ecosystem services according to the purpose of the study (Figure 2).

Tool used to spatialize ecosystem services	Description of tool
Integrated valuation of ecosystem services and tradeoffs – InVEST*	"InVEST is an open source software model used to map and value goods and services from nature in sustaining human life"
Soil and water assessment tool– SWAT**	It was developed as a conceptual and sustainable model to assist water resources managers in assessing water resources in basins.
Artificial intelligence for ecosystem services– ARIES**	ARIES provides an intelligent modeling platform that can create complex ecosystem services models.
Fog Prevention to improve stream flow in the tropics– FIESTA**	Model used for national scale fog suppression estimates and its potential impact on water resources, with emphasis on water production service. In addition, it evaluates the energy balance and horizontal precipitation.
Integrated multi-scale models of ecosystem services– MIMES	Spatialization and valuation model of ecosystem services
Co\$ting Nature**	Model of spatializing ecosystem services and prioritizing protected areas
Ecological Asset Inventory and Management– EcoAIM	A spatialization model of ecosystem services and stakeholder preference, a GIS optimization model that balances ecosystem service variables and risk analysis, including metric weighting of stakeholder preference
ECONOMETRIX	Calculates changes in ecological functions using a holistic focus that requires in situ collection (describing data) of the physical characteristics of an area
The Global Unified Metamodel of the Biosphere– GUMBO	Model used to simulate global dynamics and interactions with natural capital

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