THESIS

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THESIS TITLE

SOYBEAN RESPONSE TO WATER STRESS IN-VITRO GERMINATION

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1. INTRODUCTION

Soybean is one of the most important widely cultivated crops grown mainly for oil and food for human consumption. It is a rich source of protein and an excellent feed supplement, particularly for monogastric animals (*Montoya et al.,2017).* Though in some part of the world, soybean is not given much of attention and taken for granted unlike maize, wheat, and rice. Soybean is native to Asia dating back to the 11^{th} century BC (NCSOY, 2019) but currently grown worldwide and one of the most popularly produced crops in Brazil, United States and Argentina with 82% of the global soybean seed production (STATISTA, 2022). Over the years, there has been a significant increase in the production and the market value of soybean as it has become one of the most important commodities in global trade (Sun *et al*., 2018). World production increased from approximately 160 million tonnes on 70 million ha in 1998 to 350 million tons on 125 million ha in 2018 (FAOStat, 2021). In 2020, Global soybean production was a total of 399 million tons, of which Brazil produced 128.5 million tons (USDA 2021). Soybean is grown in Europe on 5294 thousand ha in 2020, with a production of 10,627 thousand tonnes, representing only 3% of world production (FAOStat, 2022). In Hungary, 85,440 tons of soybean was produced in 2010 and its production rose to 156,580 tons in 2021 (Statista, 2023). Soybean have a wide range of uses in human and animal foodstuff, industrial purposes and production of consumer products. According to the article published on the New Jersey Soybean Board (2022), a whole soybean is composed of 80% meal and 20% oil. 97% of the meal is used to feed poultry and livestock while the other 3% is used in food product like soybean milk. 61% of the oil is used as vegetable oil, 31% as biodiesel and 8% for industrial uses. Soybean is also used in the pharmaceutical and chemical industries. Soybean have properties that promote health as it is a very good source of bioactive peptides (Borawska, *et al.,* 2014). In the soil, soybean enhance and promote soil biological activities and help to fix nitrogen to the soil through the symbiotic relationship between the root nodules and symbiotic bacteria Bradyrhizobium. This makes its cultivation extremely useful and economical as it is considered as a means of improving soil fertility.

As with other crops, soybean growth, development and crop yield vary annually and is dependent on the various factors that affect its germination. These factors could be biotic (diseases, insect pests and weeds) or abiotic (drought, water submergence, salt, and heavy metals). Seed germination is a phase of soybean lifecycle which determine the seedling establishment and subsequent canopy growth. Germination primarily depends on environmental variables such as moisture conditions, temperature, harmful radiation and salinity. Water stress is the major environmental factor that affect soybean growth and yield as it affects its seed germination, flower production, seed number, pod growth, seed fill period and seed size (Westgate and Peterson 1993). Extreme unstable water condition may have a significant impact on the seed germination process as an optimum moisture level is required for imbibition, enzyme activation, embryo stimulation, reserve mobilization, plumule emergence and elongation. Research on the detrimental effects of soil moisture stress on soybean crop performance and yield has received more attention in recent years as it results in significant losses of soybean output (Leng and Hall, 2019). The objective of my research is to examine soybean response to water stress in in-vitro germination.

2.LITERATURE REVIEW

2.1. Origin and History of Soybean

According to the article published by the North Carolina Soybean Producers Association (NCSOY, 2019), soybeans is said to originate from Southeast Asia where it was domiciliated first by Chinese farmer around 1100BC and was grown in Japan and many other countries by the first century AD. In 1765, a colonist in the British colony of Georgia sowed the Chinese soybean seed. In 1851, soybean seeds were distributed to farmers in Illinois and the states that make up the corn belt as a gift from a crew member who was saved from a Japanese fishing boat in the Pacific Ocean. Farmers started planting soybeans as cattle forage in the 1870s, which led to an increase in their popularity. The plants thrived in the North Carolina summer's sweltering heat and humidity. The United States Department of Agriculture began testing soybeans at the turn of the century and urged farmers to sow them for use as animal feed. In 1904, George Washington Carver, an American chemist, found that soybeans are an excellent source of protein and oil. He also understood how beneficial soybeans are for maintaining healthy soil. Mr. Carver advised cotton growers to "rotate" their crops over a three-year period so that legumes like peanuts, beans, and sweet potatoes would replenish the soil's minerals and nitrogen for two seasons before cotton was planted in the third year. Many farmers were shocked to find that this resulted in a much better cotton yield than they had seen in many years. William Morse in 1919 co-founded the American Association of Soybean and became its first president as he recognized that there was more significant potential to be discovered in the soybean plant. At that time, farmers used only 20 proven varieties of soybean. William Morse spent two years gathering soybeans in China. In 1929, he came back with over 10,000 varieties of soybean for agricultural researchers to analyse as he has that understanding that improved variety translates a higher yield for farmers. In the 1940's, the farming of soybean really began in America as the soybean production in China which was the main supplier at that time was ceased by the World War II and internal revolution. When the United States entered the war, the demand for oils, lubricants, plastics, and other goods sharply soared, significantly raising the demand for soybeans. In the 1950's, the United States had a period of rising affluence after the Second World War. As people's diets improved, there was an increase in demand for meat intake. Soybean meal was discovered by livestock producers to be the preferred, cost-effective source of protein. Tens of millions of tons of soybean meal were fed to cattle, pigs, chickens, and turkeys every year. The development of the soybean to withstand herbicides in the 1990s was one of the greatest scientific achievements in agriculture. Thus, weeds could be eliminated by farmers without destroying the soybean plant. Since they wouldn't have to use steel tools to cultivate the crops, there would be less soil erosion, less fuel used, and a higher yield per plant. Because of this technology, American farmers are now able to supply to the world at a time when the demand for food is at an all-time high.

2.2. Description of soybean plant

The soybean (*Glycine max* (L.) Merr.) is an erect herbaceous annual leguminous plant which grows to up to 1 m high. On ideal soil, its branching tap-root can reach a depth of 2 m, with secondary roots penetrating the top 15-20 cm of the soil, the roots get infected with Bradyrhizobium japonicum and form root nodules while the Leaves are trifoliate with netlike veins and leaflets are oval to lanceolate (Ecoport, 2010). The papilionaceous flowers have a corolla that measures 5 to 7 mm long and are white, pink, purple, or bluish. (Giller *et al.*, 2007). Fruits are two- or three-seeded pods which developed from the flower and are characterised by spherical, yellow seeds that can range in colour from yellow to black (Koivisto, 2006). The developmental stages are blooming, formation of pod and maturity. As maturity progresses, the leaves begin to turn yellow and fall off before the pods are fully matured. Soybean varieties differ in height, from about 40cm in early types to 120cm in late-maturing types and the amount of branching also increases in longer season varieties (Da Mota, 1978). Genetically modified soybeans are now widespread in the main producing countries, and occupied 65.8 million hectares in 2008, about 68% of the world soybean area (FAO, 2010). In 2009, 91% of the US soybean surfaces were planted with GM soybeans, mostly herbicide-tolerant varieties (UDSA-NASS, 2009). The majority of the grown GM soybeans have herbicide-resistance traits. However, GM varieties have also been created for other traits, such as resistance to fungi and insects, tolerance to drought and salinity, and improved nutritional and/or health characteristics, such as high oleic content, high protein and amino acid content (especially methionine), and reduced stachyose and raffinose. An average soybean seed composition is 19% of oil, 34% of protein, 21% of fiber, 9% of carbohydrate, 4% of ash and 13% of moisture (NCSOY, 2019). Soybean is the largest oilseed crop, with 231 million tons produced in 2008, the main producers being the United States, Brazil, Argentina and China (FAO, 2010). The expression of the oil yields a high-protein cake that can be further processed into a range of products for animal feed and food purposes. While soybean used to be grown primarily for its oil, the expansion of the crop is now driven by the demand for soybean meal and feed use accounted for about two-thirds of the value of soybeans in recent years (FAO, 2006).

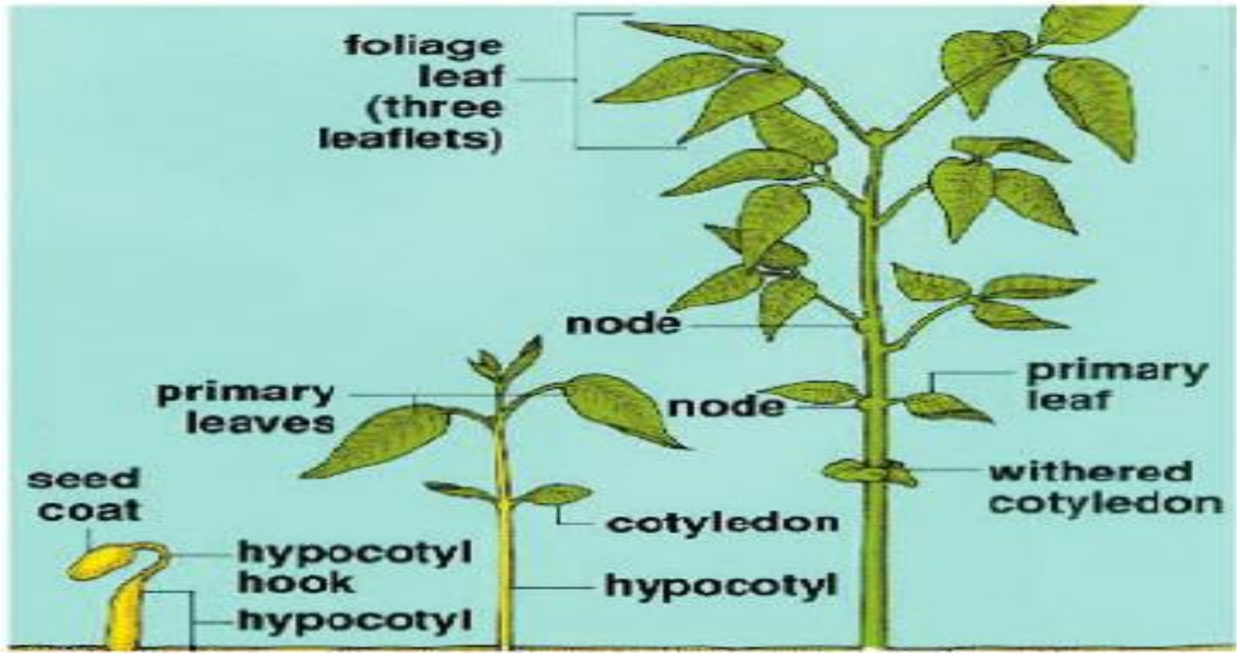

Figure 1. Morphological description of soybean plant (FAO 2006)

2.3. Soybean growth phase

A soybean seed typically takes two days to germinate after planting and about a week to sprout from the ground. According to Lowa Agricultural Literacy Foundation, up to 80 pods and between 160 and 200 seeds per plant can be produced by a single soybean plant with 2-4 pea-sized beans in each pod. The two main stages of soybean growth are the vegetative and reproductive stages. The growth stages of soybean plant were well explained in an article published by North Dakota State University.

2.3.1 Vegetative (V) Growth Stages

Vegetative growth stages of soybean through four or more stages of maturation before stage before the start of the reproductive growth stages. It is represented as V and the various stages are designated by VE, VC, V1, V2, V3 to V(n).

VE Stage

The soybean's vegetative growth stages begin with the VE stage which is the stage of emergence. Soybean germination begins when the soybean seed absorbs water equal to approximately 50% of its weight. primary root or radicle, is the first to emerge from the seed and shortly after, the cotyledons (seed leaves) are pulled along by the hypocotyl (stem) as it starts to grow toward the soil's surface. When it emerges and as the cotyledons spread out, this hook-shaped hypocotyl straightens out. In this stage, lateral roots are beginning to grow out from the primary root along with emergence of root hairs which provide the key nutrient and water-absorbing functions of the plant. Depending on soil temperature and moisture, variety and planting depth, plant emergence normally takes 10 to 18 days

cotyledon Stage (VC)

Following emergence, is the VC stage which is the Cotyledon stage when unifoliolate leaves have fully developed. At this stage, cotyledons supply the young plant the nutritional requirement for about 7 to 10 days.

First Trifoliolate (V1)

This is the stage when the first trifoliolate is fully opened and emerged. The highest, fully formed leaf node on the main stem above the unifoliolate leaves serves as a marker and identifier for the V stages after VC (the V1 to Vn stages are numbered by fully developed trifoliate). When determining V stages, only the trifoliolate leaves off the main stem are considered in the count; trifoliolate leaves on branches are not counted. Later, after V1, the plant can support itself through the photosynthesis occurring in the growing leaves. New V stages start to appear around every three to five days

Second Node (V2)

In this stage of the vegetative growth, the plant is between 6 and 8 inches tall, has two nodes above the unifoliolate node, and has unfolded leaflets on its trifoliolate leaves. Bacteria are beginning to actively fix nitrogen as the majority of these root nodules contain millions of bacteria and are located less than 10 inches below the soil's surface. At this stage, the plant will use both the nitrogen produced by bacteria and the applied or intrinsic soil nitrogen as lateral roots are forming quickly in the top six inches of soil.

Third To Fifth Nodes (V3-V5)

At the V3 stage, soybean plants are about 7 to 9 inches tall having three nodes above the unifoliolate node and will be about 10 to 12 inches tall with five nodes of fully expanded leaflets at V5. V5 stage is a stage where the plant's axillary buds in the top stem usually develop into flower clusters known as racemes. It is about one week or less from R1, or first flower. At this stage, the maximum number of nodes that the plant can produce is established.

Sixth node (V6)

V6 stage is a stage when the plants are 12 to 14 inches tall with seven nodes having leaves with unfolded leaflets. At this stage, the unifoliolate leaves and cotyledons may have fallen from the plant due to senescence and there is presence of lateral roots. Every 3 days, new V stages appear.

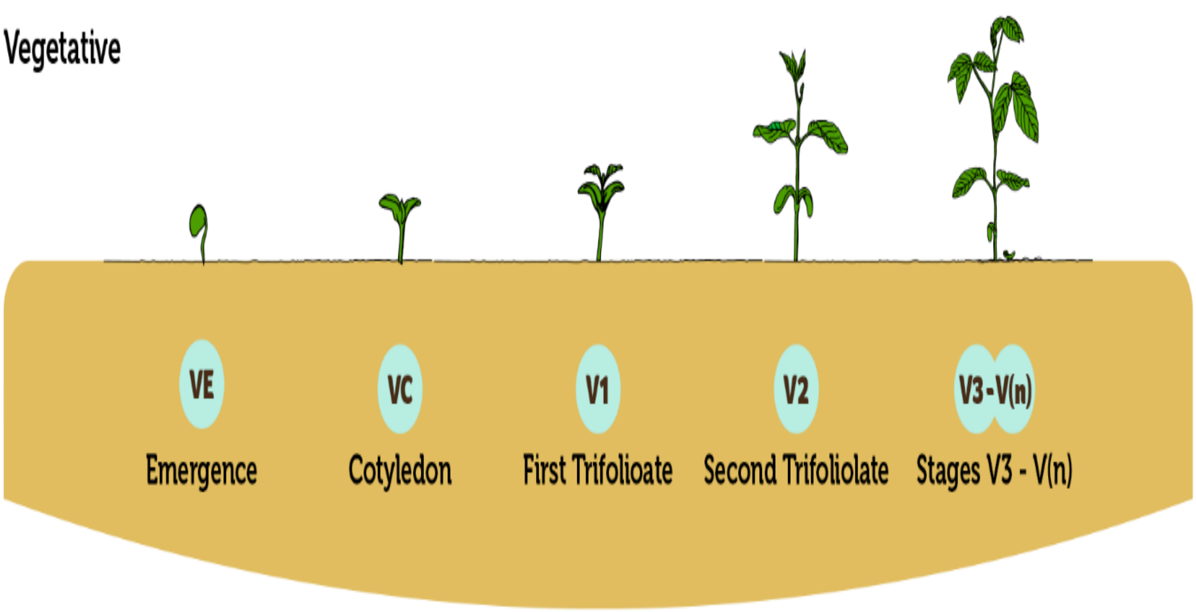

Figure 2.Typical vegetative growth stages of soybean (Source: Source: Crop nutrition. Soybean development and growth staging. www.eKonomics.com)

2.3.2 Reproductive (R) Growth Stages

The reproductive (R) stage starts at flowering and progresses to pod development, seed development, and then plant maturation. Soybean reproductive stages go through eight growth phases before attaining physiological maturity and designated by R1 through R8 with two stages describing each growth development. R1 and R2 describes flowering, pod development takes place in R3 and R4, seed development is described in R5 and R6 while R7 and R8 are the stages of plant maturation. Vegetative growth and nodal production continue through some of the R stages, including the V stage (total number of nodes fully developed).

Beginning bloom (R1)

The R1 (beginning bloom) stage is the first growth phase in the soybean reproductive stage. At this stage, the plants are 15 to 18 inches tall. Flowering begins on the third to sixth node of the main stem and few days later, flowering starts to take place on the branches. These first flowers generally appear at the base of a raceme and as the raceme elongates, new flowers begin to appear the tip of the raceme.

Full bloom (R2)

At R2 stage the plants are 17 to 22 inches tall and have reached full bloom. In this stage, several major lateral roots grow and turn downwards and along with the tap root, they continuously elongate deeply into the soil with a significant increase of nitrogen fixation.

Beginning pod (R3)

In this stage, the Plant is 23 to 32 inches tall. There may be presence of developing pods, withering flowers, open flowers, and flower buds on the same plant at this time. Developing pods are found on the lower nodes where flowering first began

Full pod (R4)

At R4 stage, the plant grows to a height of 28 to 39 inches. In this stage, one out of the four topmost nodes on the main stem with completely formed leaf bears a pod which is 3/4 inch long. During this time, pods grow quickly and seed development starts to take shape.

Beginning seed (R5)

At this stage, the plant has reached the height of 30 to 43 inches and is distinguished by quick growth of seed and circulation of dry weight and nutrient within the seeds. At the initial stage of R5, reproductive development ranges from freshly opened flowers to pods having seeds of 8 mm long. Several activities take place simultaneously at midway between the R5 and R6 stages. The plant reaching its maximum height, node number, and leaf area; high rate of nitrogen fixation and its rapid drop; and dry weight, and nutrient accumulation in the seeds are the activities that take place at approximately R5.5. A short while after

R5.5, dry weight and nutrient accumulation in the leaves, petioles, and stems reaches a peak and then start to relocate from these parts of the plant to the rapidly developing seeds.

Full seed (R6)

The height of the plant at this stage is 31 to 47 inches. At this stage, the green bean is distinguished by having a width equal to its pod cavity although various sizes of beans may be found on the plant at this time. In this stage; the weight of all plant pods seems to be the greatest.

Beginning maturity (R7)

This is the stage where the Physiological maturity of each individual soybean seed begins when the buildup of dry weight terminates. First, this happens when the seed and pod has typically lost all of its green color although some of the plant's pods still have a green tint at this stage. The plant is practically at physiological maturity because little amount of dry weight is stored. When a soybean seed reaches physiological maturity, it contains all the plant parts required to start the next generation of soybean plants and has a moisture content of roughly 60%.

Full maturity (R8)

R8 is the stage where the plant has reached it full maturity with a 95% of its pod having mature pod color. At this stage, drying weather is required for the plant to have less than 15% moisture before harvesting.

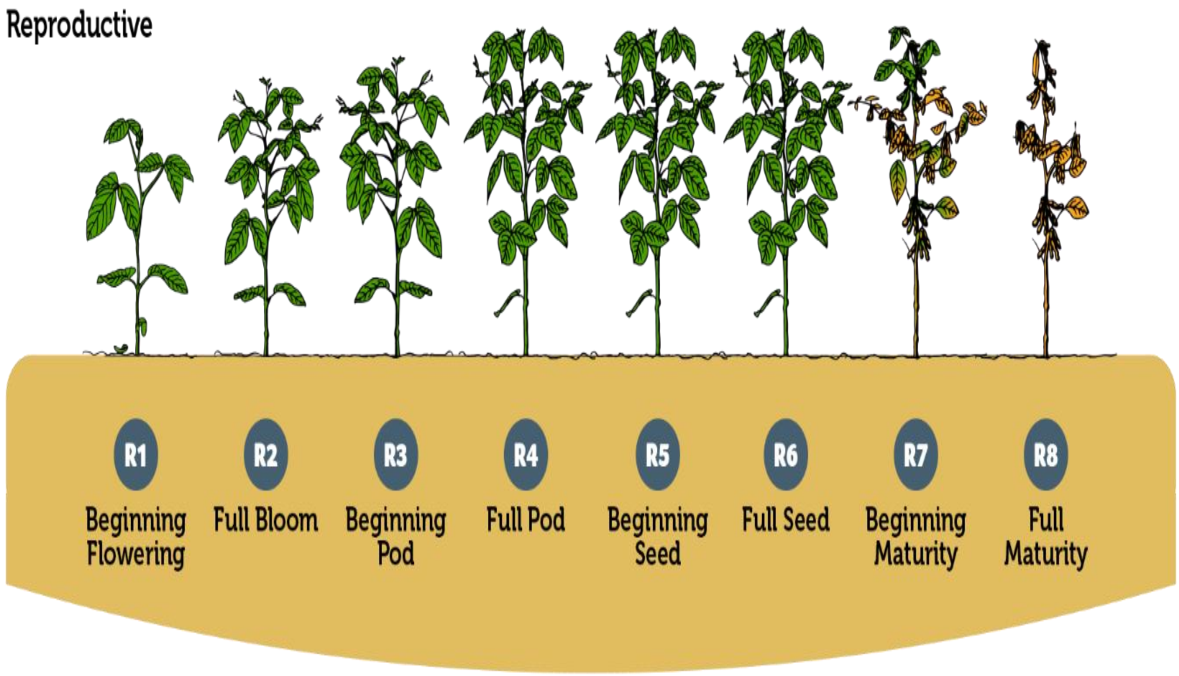

Figure 3. Typical vegetative growth stages of soybean (Source: Source: Crop nutrition. Soybean development and growth staging. www.eKonomics.com)

2.4. Water

2.4.1 Soybean water use at different stages of germination

Water is frequently the main element that limits the production and yield of soybean, making it a crucial management concern. According to FAO, Water requirements for maximum production vary between 450 and 700 mm/ season depending on climate and length of growing period (FAO, 2021). The water requirements are given by the crop coefficient (kc) in relation to reference evapotranspiration (ETo) (FAO, 2021). Soybean water use varies throughout the growing season. This variation is not only based on the climate but due to the different growth stage of the plant. Seasonal water use for soybean can range from 20 to 26 inches during the growing season (Kranz and Specht, 2012). This is dependent on planting date, maturity, growth stage, location, and weather (Helsel D and Helsel Z, 1993). Soybean plant limited access to water to meet ET demands during the crucial water consumption tends to result in significant yield decreases. 0.7 inches of water per week is required during soybean vegetative growth stage, at flowering stage (R1 and R2) twice that amount is required per week while it increases to 1.4 – 1.75 inches of water per week during pod elongation (Matcham E and Conley S, 2020). The rate of evapotranspiration during the different growth stage, determines the amount of water used by the soybean plant. The rate of evapotranspiration depends on the crop canopy's response to solar radiation, air temperature, relative humidity, and wind and as the canopy develops to about 2.5–5.0 mm per day the water usage increases (Leopold R and Olha B, 2022). According to an article by Decalb Asgrow Deltapine, the Estimates for total consumptive water use from emergence through V6 is about 3.0 inches. During these early reproductive stages, daily water use (ET) can approach 25 inches of water per day. Total consumptive water uses by the crop during R1 and R2 stages is estimated to be about 3.75 inches. During the mid- to late-reproductive stages, which includes pod development (R3 to R4) and seed fill (R5 to R6), soybean is most sensitive to water stress. On average, daily water use increases to about .32 inches per day during the R3 through R5 stages, then begins to decline at R6. In extreme cases of hot and windy conditions, daily ET may approach .50 inches per day. The total amount of water consumed from R3 through R6 may be 13.5 inches or higher (Decalb, 2015). 65% of all the water utilized by a soybean crop is expected to be used from R1 through R6 reproductive stages when the entire canopy and rooting volume have been reached (Kranz and Specht, 2012).

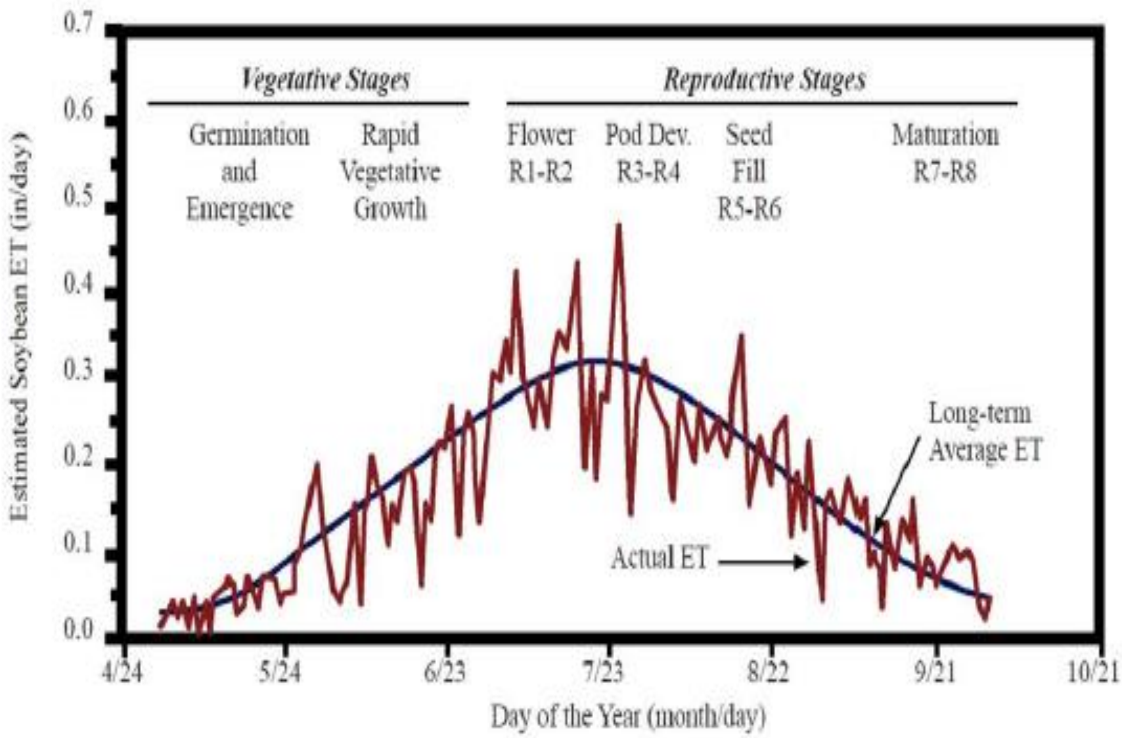

Figure 4. Example of long-term daily average and individual year soybean water use with selected growth stages. Source: Kranz, W.L. and Specht, J.E. 2012. Irrigating soybean. NebGuide G1367. University of Nebraska-Lincoln Extension.

2.4.2. Soybean water stress

Water stress tend to takes place as a result of water deficit when crop demand for water exceeds the available amount in the soil or due to poor quality of water that hamper its use as a result of water excess in terms of flooding. It is one of the environmental factors that can affect plant cell structure and function through biochemical and physiological changes (Staniak, *et al.*2023). Water deficit tends to be the main cause of water stress in crops due to the increasing climate change in recent years which has negatively affected the periodic precipitation that has led to poor yield of agricultural production. Limited access to water inhibits a number of physiological activities like photosynthesis, transpiration, and stomatal conductance as a result of water deficit in the plant tissues (Souza, *et.al,* 2013). Productivity loss as a result of water deficit is influenced by the phenological stage, the duration of the stress, and its intensity. Soybean has greater tolerance against drought stress (Marinho et al., 2022). Soybean tolerance to moisture stress is relatively high during the vegetative stages than during germination and reproduction when yield losses are most likely to (Micheal, 2020). Soybean growth phases that are more susceptible to water stress when the plant does not receive enough water to meet evapotranspiration (ET) demand are the mid to late reproductive stages.

Stress at this time tends to be the major cause of yield loss as it reduces the number of pods per plant at the point when the plants are no longer able to produce new blossoms and pods (Micheal, 2020). In research carried out by Doss *et al,* it was observed that stress administered during pod formation or pod fill caused higher yield decreases than stress imposed during floral induction or flowering (Doss *et al.,* 1974). In another study carried out in Turkey on the effect of water stress on five reproductive stages of soybean for semi-arid climatic conditions of Turkey shows that any drought stress that was imposed during R3 (beginning of pod), R5 (beginning of seed), and R6 (full seed) stages resulted in a significant decrease in yield.

2.4.3 Soybean Irrigation

Several research have sought to establish a crucial period for soybean irrigation. The quantity of water still available for plant use can be calculated by monitoring the soil water loss due to ET. Water is applied when the soil's water content reaches the maximum acceptable depletion (MAD) threshold. A MAD of 50% should often be used by growers as a threshold for starting irrigation.

Water usage by soybean during the seedling stage is extremely low. Supplemental irrigation is typically not required during the germination or vegetative growth stages unless the soil is excessively dry.

Too much water early in the season can prolong the vegetative growth stage, which can result in delays in flowering, increase plant height, and lodging (Kranz and Specht, 2012). Early season irrigation may cause a rise in the occurrence and intensity of root and crown rot diseases. Reducing watering during the early growing season can encourage soybean plants to grow larger, healthier roots that delve deeper into the soil profile. During the initial phases of growth, farmers are encouraged to rely as much as possible on moisture stored in the soil and natural precipitation. Irrigation may be required during flowering on soils with an insufficient water holding capacity (coarse soils) or when conditions are exceptionally dry (Kranz and Specht, 2012). It is crucial to provide enough water during seed fill when water is administered during flowering. This is due to the fact that irrigation during flowering typically increases the number of seeds produced, but water stress during seed fill tend to reduce the size of the seeds, which may result in greater yield lose than would have occurred if the crop had not received any water at all during flowering (Kranz and Specht, 2012). Soybeans to achieve their maximum weight, adequate water supply is required throughout the reproductive stages. This was supported in an article published in the Arkansas Soybean Production Handbook as it was recommended to irrigate the crop as needed to avoid moisture stress and to provide good soil moisture at seed fill (R5-R6 growth) stage, ensuring that the seeds achieve their maximum size (Tacker and Vories, 2000). It is estimated that soybeans require about 3.5 inches from the conclusion of seed enlargement (R6) through physiological maturity (Yonts. *et al,* 2008). Discontinuing irrigation before physiological maturity can result in yield penalties if the soil water content is not sufficient (Decalb, 2015). The effect of irrigation on oil and protein content of the grain is rather insignificant. However, under adequate irrigation there is a tendency toward a slight increase in protein content and a slight decrease in oil content (FAO, 2019).

2.5. Distribution and production

2.5.1. Production

The primary gene pool of soybean is believed to come China. In the early years, China, Japan, Indonesia and the Republic of Korea were the major producers of soybean. By 1974, nearly 63 million hectares of soybean were grown in approximately 25 countries (Da Mota, 1978). Today soybean, is produced in 46 counties (WAP, 2022). The soybean industry is highly concentrated, with cultivation primarily centered in the United States, Brazil, Argentina, China, India, Paraguay, Canada. The top three countries accounted for 80% of overall production and dominated global exports in 2022 (WAP, 2022).

According to the statistics published by M. Shahbandeh in February 2022, the United States was the leading global producer of soybeans from 2015/16 to 2018/2019 with a production volume of 120.52 million metric tons in 2018/2019. As of May 2020, Brazil overtook the United States as the leading soybean producing country with a production volume of 138 million metric tons in 2020/21 (Statista, 2021). A report released in December 2022 on the World Agricultural production website shows that in 2022, Brazil produced 153,000,000 metric ton of soybean while USA produced 116,376,000 metric ton (WAP, 2022) which is in accordance with the result published by Statista.

Table 1.Leading soybean producing countries worldwide from 2012/13 to 2022/23*(In million metric tons) (Source: M. Shahbandeh, 2023. Statista)*

Year	Brazil	United States	Argentina	China	India	Paraguay	Canada	Other
2022/23	153	116.38	45.5	20.33	12	10	6.54	24.26
2021/22	129.5	121.53	43.9	16.4	11.9	4.2	6.27	24.41
2020/21	139.5	114.75	46.2	19.6	10.45	9.9	6.36	21.76
2019/20	128.5	96.67	48.8	18.1	9.3	10.25	6.15	22.61
2018/19	120.5	120.52	55.3	15.97	10.93	8.51	7.42	23.52
2017/18	123.4	120.07	37.8	15.28	8.35	10.26	7.72	20.53
2016/17	114.9	116.93	55	13.6	10.99	10.34	6.6	21.42
2015/16	96.5	106.87	58.8	12.37	6.93	8.86	6.46	19.12
2014/15	97.2	106.89	61.4	12.15	8.71	8.15	6.05	19.2
2013/14	86.7	91.39	53.4	11.95	9.5	8.2	5.4	16
2012/13	82	82.79	49.3	13.05	12.19	8.2	5.09	15.96

2.5.2. Average yields

Soybean yield is not stable. It is dependent on water availability, fertilization and row spacing. According to FAO, under rainfed conditions, good soybean yields vary between 1.5 and 2.5 ton/ha seed. High yields of improved varieties are between 2.5 and 3.5 ton/ha seed under irrigation. The average soybean yield per acre is 49.8 bushel (USDA, 2022). Average soybean yield for the farms in 2016 to 2020 was 2.79 metric tons per hectare (41.4 bushels per acre). Average farm yields ranged from approximately 1.48 metric tons per hectare for the typical farm in Russia (21.9 bushels per acre) to 3.94 metric tons per hectare for the typical farm in west central Indiana (58.6 bushels per acre) (Agri-benchmark, 2022). Brazil yield increases from 3.1 ton/ha in 2021/2022 to 3.5 tons/ha in 2022/2023 (52.6 bushel per acre) (Maples, 2022). Soybean production for 2022 totalled 4.28 billion bushels, down 4% from 2021. The average soybean yield is estimated at 49.5 bushels per acre, 2.2 bushels below 2021, and 0.7 bushel (USDA, 2023).

2.6. Uses

Soybean is a unique plant that can be used as a beverage & food, animal feed as well for industrial purposes. As a beverage & food, it can be used in1 making milk, yoghurt, flour and protein powder which is prominent in Nigeria, it also serves as an alternative for meat and for making soy sauce. The oil is used for cooking. As an animal feed, it is processed into soybean meal which is used in feeding poultry and livestock. It is also used in making pet food. Industrially, soybean is used in making biofuel, lubricant, tires, plastics, adhesive and paints. In the early 50's, Henry Ford took a bag of soybean to his laboratory and the scientists in his lab made a soy-based plastic which was strong enough for the gearshift knobs, horn buttons, window frames, accelerator pedals, light switch assemblies and ignition-coil casings and by 1953, Ford was using one bushel of soybean for every car he manufactured (NCSOY, 2019).

3. MATERIAL AND METHODS

3.1: Field experiment

The first trial for the germination test was carried out at the University experimental field. The Hungarian soybean variety was sown. After sowing, it was irrigated immediately with subsequent irrigation with the first five days but it yielded no result as no seed germinated. This was due to the extreme summer drought that year.

3.2: First laboratory experiment

The second trial was carried out in the laboratory of crop production in the Faculty of Agriculture and Environment at Hungarian University of Agriculture and Life Sciences (MATE) Gödöllő Hungary following the standard laboratory procedure. The same Hungarian variety was used for the trial in nine treatments with 4 replications and it yielded no result as no seed germinated.

3.3: Similar experiment in the laboratory

A similar experiment was conducted by Kiet Anh Huynh few months earlier. The trial was carried out in the laboratory of crop production in the Faculty of Agriculture and Environment at Hungarian University of Agriculture and Life Sciences (MATE) Gödöllő Hungary. This trial serves as the bases of my evaluation. As already mentioned above, the objective was to investigate the response of soybean to water stress in invitro germination. To do that, the following materials were used:

- Transparent petri dishes
- Micro pipette
- Filter paper
- Distilled water
- Soybean seed
- Paraffin wrap

3.4. Soybean seeds used for the experimentation

Under these experimental settings, three replications of Hungarian hybrid seed samples were examined for viability, radicle, and plumule following the general laboratory standards.

3.5. Abiotic stress treatment

During the experiment, soybean seeds germination was tested under water stress as an abiotic factor. The treatments were set in a randomized complete block design (RCBD), having 3 repetitions. The same size and same depth petri dishes were used. One white round filter paper with the same size as the petri dishes were placed in each petri dish. The trial set was 3 treatments with 3 replications.

3.5.1. Water treatment

Water is a crucial component for seed germination. In this trial, Petri dishes were used to evaluate three different water quantities in each soybean seed. The following were the treatments:

Water treatment 0: seeds soaked in 6 ml of water,

Water treatment 1: seeds soaked in 9 ml of water,

Water treatment 2: seeds soaked in 12 ml of water.

3.6. Manipulation

During the experiment, healthy and uniform in size soybean seeds were used. The were put inside each petri dish, as the figure 3 showed. All seeds were treated with fungicide to eliminate pest factor to the germination of soybean seed before realizing the experimentation. In total, 6 petri dishes were used containing 10 seeds in each petri dish. Afterwards, 3 levels of water (6 ml; 9 ml; 12 ml) were added in each dish using micropipette and were left in a climate chamber for one week to provide the seeds enough time for germination.

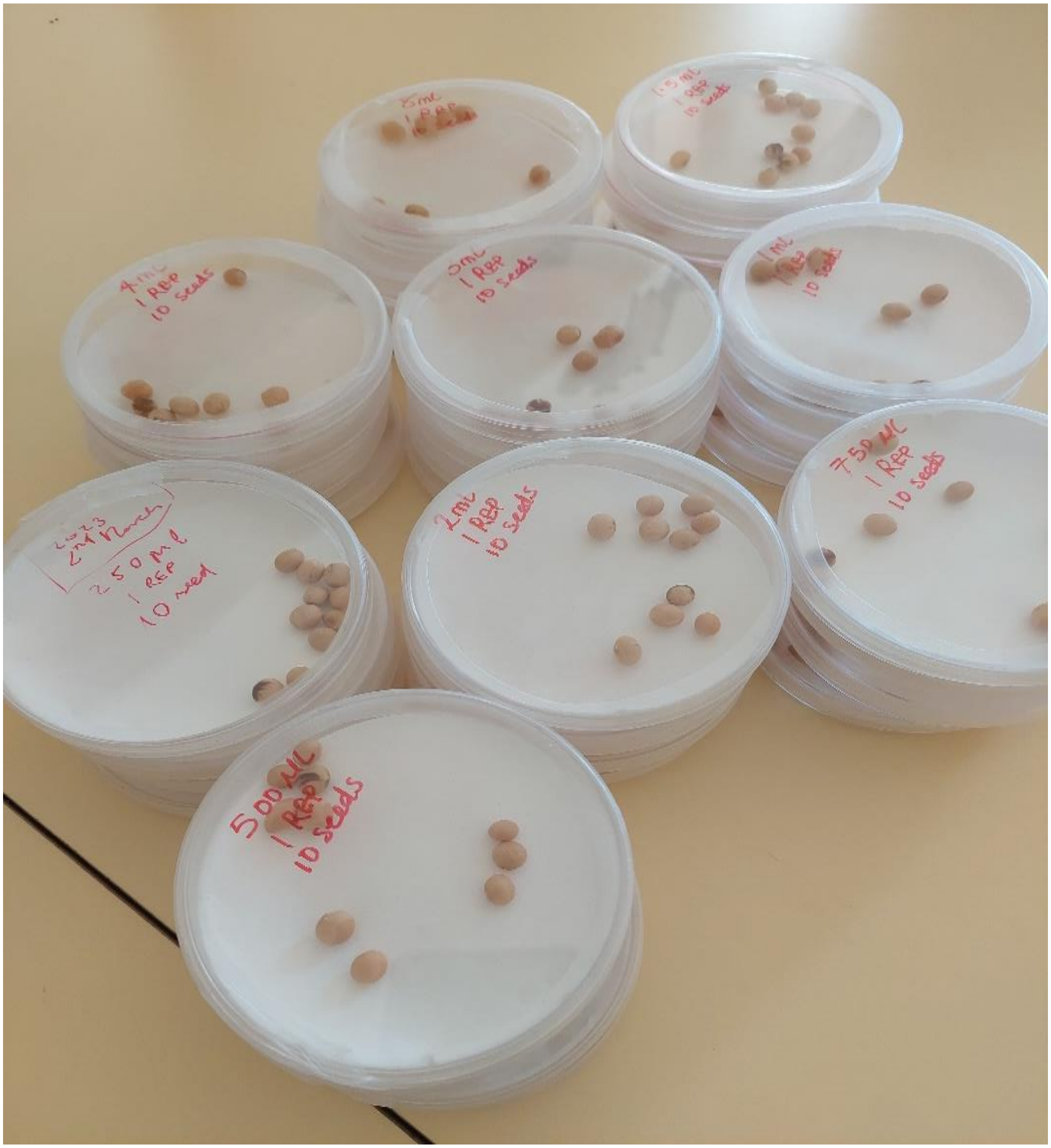

Figure 5. Micropipette, seeds, dish petri used during the experiment.

3.7. Collection of the results and data

After one week or 7 days, which is enough to wait for the seeds to germinate, they were removed from the chamber climate and the number of the following parameters were recorded:

- Number of germinated seeds
- Radicle length
- Plumule length

3.8. Data analysis

For the statistical evaluation of the results, Explore and ANOVA modules of the IBM SPSS V.23 software were used. The effect of the different treatments on the seed germination was analysed using one-way ANOVA at a 0,05 level of significance. Values are given as the mean ± standard deviation of four measurements. LSD (least significant difference) tests were used to determine the significant difference among data. The statistical significance level was $p<0,05$.

4. RESULTS AND DISCUSSION

This part compiles the main results, analyses and discussion of the recorded data and observation obtained from the experimentation. Moreover, in this part, the effect of water stress on soybean seed germination of the Hungarian hybrid seed is presented.

4.1. Effect of different water levels on soybean seeds germination

In the experiment three levels of water supply were used in an experiment on water stress. According to this study, the two levels of water, 6 ml and 9 ml, had the highest soybean seed germination ratios, with seeds germination percentages of 40.74 and 22.22 for 12 ml of water, respectively. Waterlogged soybean seeds, however, were prevented from further germination and development after a few days at the 12 ml water treatment (Fig. 6).

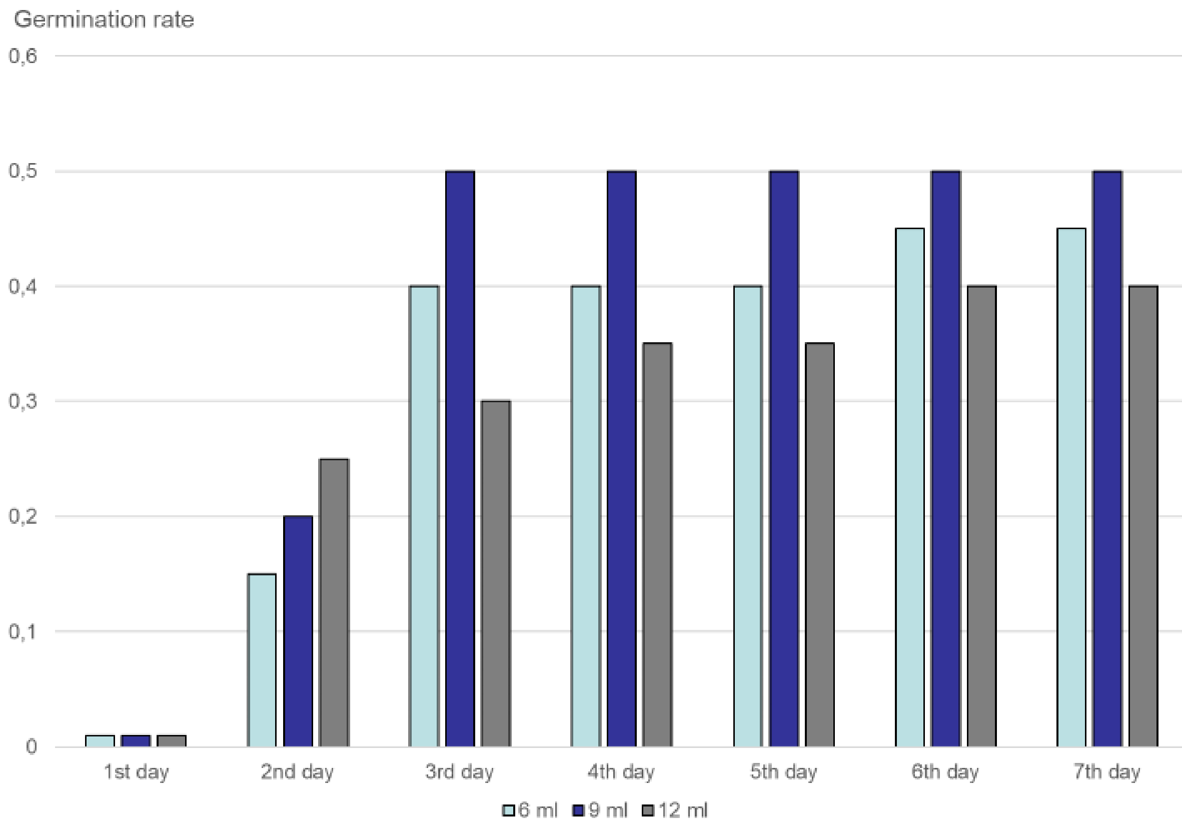

Figure 6. Germination response of soybean seeds to water level. Each point represents a mean (n=3).

The effects of water levels of 6 ml, 9 ml, and 12 ml on soybean seed germination were compared using a one-way ANOVA between treatments (Table 2). There was a substantial difference between the effect of water levels on the radicle length and plumule length of seeds that germinated.

Table 2. Summary of one-way Anova showing a degree of freedom (Df), F, and probability for each analysis under water stress conditions. Significant P-values are highlighted in bold.

Parameters	Df	F	P
Germinated Seeds	80	0,338	**0,714**
Radicle length	80	3,495	**0,035**
Plumule length	80	2,226	**0,115**

Results of a post-hoc comparison between seeds treated with three levels of water in soybean seeds are summarized in Table 3. The percentage of seeds that germinated in each treatment did not differ significantly from one another.

Table 3. Posthoc comparisons of treatments in the case of different levels of amount of water in the total of germinated seeds, using LSD. Mean differences are shown.

	6 ml	9 ml	12 ml
6 ml	-		
9 ml	-0,074	-	
12 ml	0,037	-0,111	-

**. The mean differences were not significant at 0.05 level*

Table 4. presents the comparison results of post-Hoc between seeds treated with three levels of water in germinated soybean seeds with the radicle length. There was a significant difference in the length of radicle between the 9 ml water level and the treated seeds by 12 ml of water, which was about -0.444 at p=0.031, and between the seeds treated with 9 ml and 6ml of water, which is about 0.481 at p=0.020. The length of the radicle was decreased when the soybean seeds were germinated under water stress conditions (6 ml and 12 ml). The length of the radicle of the seeds that germinated was 0.44 mm, 0.93 mm, and 0.48 mm at water concentrations of 6 ml, 9 ml, and 12 ml, respectively.

Table 4.Posthoc comparisons of treatments in the case of different levels of amount of water in the length of the radicle, using LSD. Mean differences are shown.

	6 ml	9 ml	12 ml
6 ml	-		
9 ml	0.481*	-	
12 ml	0,037	-0.444*	-

**. The mean difference is significant at 0.05 level*

Table 5. show the Post Hoc comparison results between seeds treated with three different levels of water in germinated soybean seeds with the length of plumule. There was a significant difference between the two levels of water 9 ml and 12 ml. At p=0.040, the difference was around -0.459. In condition 12ml, the length of the plumule decreased by about 49.5%.

Table 5.Posthoc comparisons of treatments in the case of different levels of amount of water in the length of the plumule, using LSD. Mean differences are shown.

	6 ml	9 ml	12 ml
6 ml	-		
9 ml	0,293	-	
12 ml	-0,167	-0.459*	-

**. The mean difference is significant at 0.05 level*

There was no difference in the soybean seed germination ratio between the levels of water. On the other hand, the waterlogging condition damaged the soybean seedling 7 days after sowing at a water level of 12 ml. Similar results were seen in a long-term waterlogging experiment with pea crop seeds (Zaman et al., 2019), where the lack of oxygen led to membrane disintegration and cellular content leakage, which resulted in seed mortality and/or unsuccessful germination. According to Carrera et al. (2021), soybean plants under drought stress suffered future yield losses. In our trials the ability of soybean seeds germination was not significantly affected by the three levels of water (6 ml, 9 ml, 12 ml).

4.2. New Result and Discussion

Effects of water stress on soybean seed germination was studied in the lab by growing the seeds in a petri dishes. The soybean seed germination ratio showed no significant differences between the three levels of water. However, the water stress treatment 6 ml inhibited the increase in the length of the radicle and this response may be because water stress has impacted the processes of metabolism within the seed. Water stress prevents seeds from absorbing water, which slows down their internal metabolic processes. As a result, the radicle will need more time to emerge from the seed, which will result in a longer germination period and a lower germination rate (De and Kar, 1994, Kiet et al 2022). 9 ml water treatment shows to be most favourable as the seeds germinated with a higher increase in length of radicle and plumule. When sufficient water (approximately 50% of the seed's weight) is imbibed and with favourable temperatures, the radicle breaks through the seed coat and rapidly develops into the primary seedling root (Decalb, 2019). On the other hand, seeds treated with 12 ml water level were waterlogged. The Waterlogged soybean seeds, however, were prevented from further germination and development after a few days. Soybean is vulnerable to the abiotic stress of waterlogging during germination, early vegetative, and early reproductive stages. Orchard and Jessop (1984) reported that decrease in seed germination ability have been attributed to a shortage of oxygen due to waterlogging. Under this stress, lack of oxygen brought on by waterlogging is the reason why waterlogged seeds of 12 ml treatment had no further germination and development after few days.

- Waterlogging conditions have obstructed germination after the second day, while normal and abundant but no flood water supply applications contributed to higher viability.
- The initial growth of the plantlets had shown significant differences in relation with the moisture conditions.
- Waterlogging had a deteriorating impact on the development of the growth of organs.
- Plumule growth was blocked significantly more than that of the radicles.
- The stress processes were detected, and identified, however more detailed physiological observations are needed in the future to specify stress conditions.

5. CONCLUSIONS AND RECOMMENDATIONS

The results of the trial obtained suggest, that abiotic stress conditions caused changes of various magnitude in the viability and initial growth and development of soybean seeds and sprouts. Stress factors such as resource depletion or excess can obstruct the physiological processes of plants, impair their metabolism and destroy cellular structures, resulting in stunted growth of plant and contributing to reduced yield and impaired yield quality. Under abiotic stresses, reactive oxygen species (ROS) are released, which are by-products of altered aerobic metabolism. ROS injure cells when they are present in high concentrations, but they function as secondary messengers in intracellular signalling cascades when present in low and moderate concentrations. In this research, waterlogging conditions prevented germination after the second day, a lack of water caused growth to be delayed, and normal and adequate water supply applications—but not floods—helped to increase germination. The plantlets' early development had revealed notable variations in response to the moisture conditions. Waterlogging had a deteriorating impact on the development of the growth of organs. In all treatments, plumule growth was substantially more effectively inhibited than radicle growth. The stress processes were detected and characterized; however, the increasing demand for food from a growing world population requires significant improvements in agricultural productivity. Therefore, future research should be directed towards enhancing tolerance to stress factors by exploiting ROS-related pathways and identifying additional roles for ROS in plant acclimatization to abiotic stresses. Knowing how plants react to stress factors and how they respond to stress is of great cognitive and practical importance. Numerous studies are testing ideas that can increase plant tolerance to abiotic stresses. The development of breeding programmes and innovations in agronomic practices can benefit soybean production.

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SUMMARY

Thesis title: SOYBEAN RESPONSE TO WATER STRESS IN INVITRO GERMINATION

Author: Ezinne Sarah Otisi

Course: MSc Crop Production Engineering

Institute/Department: Agronomy Institute

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2. Independent consultant (name, position, company) Dr. Márton Jolankai, Professor, Institute of Agronomy

Soybean is one of the most important widely cultivated crops grown mainly for oil and food for human consumption. It is a rich source of protein and an excellent feed supplement. Soybean growth, development and crop yield vary annually and is dependent on the various biotic and abiotic factors that affect its germination. In the present research work, we aimed to carry out in vitro experiment the response of soybean seed germination to water stress as an abiotic factor. To do this, the Hungarian hybrid seed samples were first sown at the University experimental field. After sowing, it was irrigated immediately with subsequent irrigation within the first and second week but it yielded no result as no seed germinated. This was due to the extreme summer drought that year. The second trial was carried out in the laboratory of crop production in the Faculty of Agriculture and Environment at Hungarian University of Agriculture and Life Sciences (MATE) Gödöllő Hungary following the standard laboratory procedure. The same Hungarian variety was used for the trial in nine water treatments with 4 replications and it yielded no result as no seed germinated. A similar experiment carried out by Kiet Anh Huynh using the same Hungarian hybrid variety formed the bases of my evaluation. The study examined the viability, radicle, and plumule growth in three-levels of water treatments (6 ml; 9 ml; 12 ml) following the general laboratory standards and was conducted in the laboratory of crop production in the Faculty of Agriculture and Environment at Hungarian University of Agriculture and Life Sciences (MATE) Gödöllő, Hungary. The treatments were set in randomized complete block design with three repetitions and the parameters recorded from the experiment were Germinated seeds, Radicle length and Plumule

length. ANOVA one way and LSD tests using the SPSS software were used to analyse the significant difference between seeds for each water level treatment. Our results showed a significant difference at the level of $p<0,05$. The two levels of water, 6 ml and 9 ml, had the highest soybean seed germination ratios, with seeds germination percentages of 40.74 and 22.22 for 12 ml of water, respectively. The percentage of seeds that germinated in each treatment did not differ significantly from one another.

A substantial difference between the effect of water levels on the radicle length and plumule length of seeds that germinated was observed. There was a significant difference in the length of radicle between the 9 ml water level and the treated seeds by 12 ml of water, which was about -0.444 at $p=0.031$, and between the seeds treated with 9 ml and 6ml of water, which is about 0.481 at $p=0.020$. The length of the radicle was decreased when the soybean seeds were germinated under water stress conditions (6 ml and 12 ml). The length of the radicle of the seeds that germinated was 0.44 mm, 0.93 mm, and 0.48 mm at water concentrations of 6 ml, 9 ml, and 12 ml, respectively. The length of plumule showed a significant difference between the two levels of water 9 ml and 12 ml. At $p=0.040$, the difference was around -0.459. In condition 12ml, the length of the plumule decreased by about 49.5%. Our result showed soybean growth is greatly affected by water stress. Under water deficit, the growth and emergence of the plumule is retarded while under waterlogged condition, there is a slow down in the germination process due to the lack of oxygen to facilitate the metabolic activities and processes within the seed.

- Waterlogging conditions have obstructed germination after the second day, while normal and abundant but no flood water supply applications contributed to higher viability.
- The initial growth of the plantlets had shown significant differences in relation with the moisture conditions.
- Waterlogging had a deteriorating impact on the development of the growth of organs.
- Plumule growth was blocked significantly more than that of the radicles.
- The stress processes were detected, and identified, however more detailed physiological observations are needed in the future to specify stress conditions.

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Thank you

Ezinne Sarah Otisi

APPENDICES

Land preparation and sowing of soybean

STUDENT DECLARATION

Signed below, Ezinne Sarah Otisi, student of the Szent István Campus of the Hungarian University of Agriculture and Life Science, at the BSc/MSc Course of Crop Production Engineering declare that the present Thesis is my own work and I have used the cited and quoted literature in accordance with the relevant legal and ethical rules. I understand that the one page-summary of my thesis will be uploaded on the website of the Campus/Institute/Course and my Thesis will be available at the Host Department/Institute and in the repository of the University in accordance with the relevant legal and ethical rules.

Confidential data are presented in the thesis: yes no*

Date: 2nd May 2023

Student

SUPERVISOR'S DECLARATION

As primary supervisor of the author of this thesis, I hereby declare that review of the thesis was done thoroughly; student was informed and guided on the method of citing literature sources in the dissertation, attention was drawn on the importance of using literature data in accordance with the relevant legal and ethical rules.

Confidential data are presented in the thesis: yes no *

Approval of thesis for oral defense on Final Examination: approved not approved *

Date: 2nd May 2023

Tamara Aros

Signature