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Evaluation of buckwheat seed vigor by analysis of residual chlorophyll in seeds

Diploma Thesis

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DECLARATION

I hereby declare that this Master's Thesis titled "Evaluation of buckwheat seed vigor by analysis of residual chlorophyll in seed" is my own work completed with the expert guidance of my thesis supervisor and consultant. I worked separately under the supervisor of the thesis and was using literature and other information.

In Prague,23/07/2020

Signature

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Summary

The history of agriculture begin during Neolithic era which happened around 9000 to 10,000 years ago. And buckwheat is one of the oldest cultivated crops through out the globe. It has remained minor neglected crops until recent decade, research and studies focused on improvement and development of cultivars. Its demand has increased over last decade due to its nutritional value, short life-cycle and hardiness.

The main aim of this thesis is to evaluate the buckwheat vigor by analyzing the residual chlorophyll content in the seed. We conduct chlorophyll content test in six variety of buckwheat using the equipment called mobile chlorophyll analyzer and observed the seed germination of different chlorophyll content in same and two different temperature in seed germination chamber.

Another aim of this thesis is to explain the buckwheat seed vigor by performing standard germination test under different combined conditions like two different temperature with two different water content. This test is carried out with twelve buckwheat variety in seed germination chamber and correlate its value with germination test value of residual chlorophyll content seeds.

We found that seed with low chlorophyll content have high germinate rate and speed, cotyledons count and better seedling stand than that of seed with high chlorophyll where germination is less and speed is low with many anomalies. We found in another test that germination speed is significantly affected by temperature. Higher temperature(20C) of our test is prove to have greater germinate of seed in short period of time than low temperature (15C). And we also observed that germination rate depends on seed breeds, some breed of seed perform well in all of the test where some of them perform bad in all tests. Additional we didn't found any significant influence by different volume of water on seed vigor testing.

Key-words

Buckwheat seed, vigour, chlorophyll content, germination, temperature difference, different water content

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1. Introduction

Agriculture had began around 9500 BC in various part of globe. Mankind have depend on large number of cultivated species for various purposes. Historically agricultural exploration has focused on few primary crops which supplies majority of food crops. Due to this, minor crop species are not given that much of attention and importance for their research and development. These minor neglected species adapt well in different growing conditions where major staple crops find hard to grow and are consider as major crop at regional level through out the world.

Buckwheat is one of the oldest cultivated and hardy plant species which thrives well in different geographical location with less care. Buckwheat are fast growing short seasonal crops which reach maturity with in 90 days. Due to their fast growth ability and short time they are use as weed suppressor and also use as cover crops. Moreover, buckwheat is major crops for in some part of the world and contributes as major source of food supply for certain duration. Due to lack of research, there is limited information available which hampers the understanding of the genetics and developing its potential. Buckwheat has been neglected for many years, it was used mostly as cover crops and fodder for livestock. But recent studies revels many of its nutritional potential and other uses. There has been surge on demand of buckwheat which lead to more land used for cultivation through out the world in recent decade due to its high nutritional values.

Recent agricultural and horticultural advancement and development were focused on refining the seed vigor to increase yield of cultivated crops. Seed vigor is a crucial aspect of seed quality.As vigorous seed tend to perform well in both suitable and unsuitable condition than seed with low vigor. Seed vigor does not have any universally accepted definition and perfect testing method. In a nutshell we can say that seed vigor means sum of all properties which checks the degree of potential of germination and viability to understand seed and seed lot activity in storage or in field. Commonly practice seed vigor testing methods are Tetrazolium test, Cold test, Paper piercing test, Growing test, Ageing test, Conductivity test. And least known and new vigor testing method residual chlorophyll content test which is done by measuring the chlorophyll content in seed.

2.Hypothesis and objectives

Seed vigor is a sum of all the connection properties and the independent potential of seed such as germination and germination speed, seedling stand, germinating capacity above and below temperature and resistant to stresses. Seed vigor is an important aspect of seed quality. There are several types of seed vigor testing methods. The main aim of this thesis are

1. To evaluate buckwheat seed vigor by analyzing the residual chlorophyll content in seed by correlating with standard germination test, to evaluate the seed performance in two different temperatures.

2. Standard germination test to evaluate seed performance of various cultivars.

The main objective is to determine germination of seed under the influence of residual chlorophyll content of seed. The study is focused on performance of different chlorophyll content seeds under different condition to see how they perform. It is assumed that seed with low chlorophyll content have high vigor than that of seed with high chlorophyll content. This study is subjected to find a different method to evaluate the seed vigor.

Hypothesis

The seed vigor determined by standard methods correlates with the residual chlorophyll content of the seeds of buckwheat.

3. Literary overview

3.1 Seed Germination

Germination of seed can be defined as the vital course during which root breakout of seed coat and sprout appears causing single seed into a plant. Crop harvest and quality is greatly influenced by germination. Seed germination order differs according to the origin of seed, parent health, environmental condition and maturity at harvest (Chaisurisri et al. 1992). Usually angiosperm reproduces through sexual reproduction and produce seeds. A determining character for the reproduction of plant species is good seed germination and formation of healthy seedlings. Germination is composed of many systematic processes which occurs when suitable conditions are meet, eventually resulting in creation of new plant. Whereas under adverse condition seeds go through dormancy (a condition in seed due to evolution for survival, which prevent seed from germination during unfavorable environmental condition which lead to low chance of seedling survival). Due to its high susceptibility to infections, damage and various other stresses, germination is regarded as the most critical stage in the plant life cycle. During germination process under optimal temperature seed quickly uptakes water, cause enzyme to activate and softening seed coat. Then radicle is appear by breaking seed coat to form root to extract water. Next, seed internal physiology triggers and starts to metabolize reserved food, respire and create proteins which help shoot growth towards the surface. Ultimately, cells lengthen and multiply which emphasize root growth and expansion of cotyledon to form true leaves, where it starts harvesting energy from sun. This is final stage of germination.

3.2 Seed vigor and its testing

Seed vigor is a crucial framework which is evaluated by germination and viability test to understand seed lot performance in storage or in field. Seed testing primary objective is to build the standard level for seed. It is a kind of concept that has particular character of seed where various aspect like; genetic element, health of mother plant, environmental factor, harvesting process, maturity during harvest, seed integrity and aging and diseases are identified to influence seed vigor. According (Delouche1980) High moisture and high temperature condition during later stage of seed maturity and development adversely effect on production of seed and cause rapid deprivation in seed vigor. Seed vigor means the property of seed showing its ability for germination, field emergence and store ability of seed under diverse circumstances than standard germination. Moreover, according to ISTA seed vigor is defined as the total characteristics of seed which checks the level of energy and performance of seed or its lots during seed germination and seed emergence under different conditions. Due to little knowledge about seed vigor and many definition by various resources, its doesn't have one definition. Rapid and uniform emergence and maturation of seed is considered as seed with high vigor where as seed with low vigor is highly susceptible to different adverse condition which lead to death of plant, diseases, less yield and low quality crop production. (Isley D.1957) has classified vigor testing into two types:

3.2.1 Direct Test:

This test is carried out by stimulating relevant unsuitable field atmosphere which at the same time calculate all vigor aspects.

3.2.2 Indirect Test:

This test evaluates particular character of seeds which allow control over variables permitting to reproduce results. They are less complicated, time taking and easier to operate than direct test. Indirect test is further categorizes into four groups.

Tetrazolium Test (Biochemical Test): Salt of tetrazolium leave red marks in all living tissues of seed embryo which tell the viability of seed. Careful inspection of staining marks in tetrazolium test informs weakness of seed which are not distinguished in standard termination test.

Stress tests: This test comprises of many individual tests like; tests under unsuitable temperature, moisture, atmosphere (exposing to vacuum), treating with chemical (hydrogen-peroxide), mechanical obstacles.

Growth Test: This test is based on the principle that seed with high vigor metabolize, germinate and stand in field under favorable condition than seed with low vigor.

Conductivity (Physical measurement) Test: This test is based on the principle of measurement of membrane permeability which mean seed with high vigor has intact cell membrane and don’t loose it essential water soluble components like sugar, electrolytes, amino acids when immersed in water where as seed with poor vigor has weak and permeable cell membrane which cause leakage of water soluble component which affect on germination. The electrical conductivity and the germination percentage of broad beans and peas has been positively associated (Matthews S. and Powell A. 1981)

3.3 History of buckwheat cultivation

Buckwheat is one of the oldest domesticated and cultivated crops in the world. Buckwheat is thought to be originated in southeast Asia around 5000 to 6000 years ago and china have been noted to farmed buckwheat as early as 1st to 2nd centuries BC (Li and Yang 1992) and the report on surge in production of buckwheat was appeared in 5th and 6th centuries AD (Krotov 1963).Roughly a millennium of cultivation in china it reached to Europe by Siberia in the mid ages, stretching upto Germany early in 15th century (Hughes and Hensen 1934). And in the 1600s it was finally introduced in North America due to movement of immigrants from many countries and mostly used on recently claimed area. (Nagatomo 1984) suggested with archaeological proof that buckwheat is considered to have been brought into Japan around 3000 years ago and first reported in 8th century in Japan as largely farmed catch crops. Buckwheat spread to japan via the Korean peninsula from northern part of china (Nagatomo 1984) and (Ohnishi 1995). And according to Jomon period cultivation of buckwheat in japan was traced back to 6600 BP (Tuskada et al. 1986).Common buckwheat is found cultivated in altitude roughly ranging from 500 to 2500 m and as it goes above 2500 m tartary buckwheat is cultivated mostly (Ohnishi 1988). Buckwheat is a dicotyledonous pseudo-cereal that resides in the Polygonaceae family and the genus of Fagopyrum. There are around 15 to 16 species of buckwheat existing in the genus Fagopyrum and are found in temperate areas of Euro-Asia (Ye and Guo 1992). Of which two domesticated species of buckwheat *Fagopyrum esculentum* and *Fagopyrum tataricum* are primarily cultivated in the northern hemisphere periodically, being a major staple in some areas in the Himalayas and in china. While others are mainly found in the high areas of Euro-Asia (Baniya 1994, Rana 2004, Ohnishi 1995).

3.3.1 Origin of species

Due to lack of strong evidence and proper research on the origin of buckwheat species controversial speculation is done. De Candolle (1883) believed that Siberia or region around Amur river as the origin place of wild *fagopyrum*, being based on philological consideration of Russian taxonomists (Ledebour 1841, Maximowicz 1859),and considered that the *Fagopyrum cymosum* a perennial species was the wild parents of common buckwheat and tartary buckwheat(Campbell 1976).

Several botanical explorations plus the historical evidence were taken into account in china, around mid of the 19th century by European scientists. Steward (1930) outlined the classification and distribution of buckwheat being southern china which is then put forward by Nakao (1957) for the origin of common buckwheat.(Komarava,1938; Krotov,1960) Russian scientist after finding the original hypothesis of cultivated buckwheat being the Northern china- Siberia not true, they insisted that Himalayan hills or Tibet to be the place of *Fagopyrum* origin. (Ohnishi 1998) Altogether added six new species, *Fagopyrum. homotropicum, Fagopyrum. pleioramosum, Fagopyrum capillatum, Fagopyrum. callianthum, Fagopyrum. rubifolium and Fagopyrum. macrocarpum* and one subspecies *Fagopyrum. esculentum* ssp. *ancestrale* which were discovered in the Yunnan and Sichuan province of China and report that *Fagopyrum. esculentum* ssp. *ancestrale* from Yongsheng district of Yunnan province is the wild ancestor of the cultivated common buckwheat, which is done studying by molecular phylogenetic analysis and by isozyme analysis along with morphology. And for the farmed Tartary buckwheat (Ohnishi 1998), put forward the little known subspecies *Fagopyrum. tataricum* ssp. *potanini* being the wild ancestor cultivated Tartary buckwheat which was discovered by (G.N Potanin) a Russian scientist on the Gansi, a Chinese province. RAPD (random amplified polymorphism DNA), AFLP (amplified fragment length polymorphism) analysis and extensive research on phylogenetic and taxonomic relationships among different species, which clarify the distribution of wild Tartary buckwheat (Ohnishi 1998).

Fig.1 Detail evaluation of phylogenetics and distribution of Tartary and common buckwheat and other species is eastern Tibet and Sichuan and Yunnan provinces. Source of Figure.https://www.semanticscholar.org/paper/Origin-of-cultivated-Tatary-buckwheat(Fagopyrum by Tsuji Ohnishi

3.4 Past, Present and Future prospective of Buckwheat

Buckwheat is a very strange and uncommon crop whose grains belong to cereal crops because of their close resemblance in chemical constitution and usage though it is pseudocereal and has different growth habit than that of cereal crops(Campbell 1997). Buckwheat is a short seasonal crop, does well in low fertile soil, acidic soil and doesn't require special care like the use of pesticides and fertilizers. Furthermore, buckwheat needs less water to grow and takes up fewer nutrients from the soil than other primary crops. (Li and Zhang 2001). Buckwheat is the world's first crop to be cultivated. Due to the imperfect reproductive organ, fertilization failure and seeding failure in the post-zygotic stage (Adachi, 1986), breeding and production are rather difficult in buckwheat so Polish Professor Marek Ruszkowaski made some effort to reproduce homostylus varieties of buckwheat to achieve buckwheat of inbreed lines which are less relied on pollination. But the population of such homozygote self-pollinating buckwheat variety will lose the potential for crossbreeding to produce high vigor offspring.

3.4.1 Past State

For more than a hundred years buckwheat is usually cultivated in mountains by the farmers with limited resources that provide food and support for people living there. Because of its unique growth habit, buckwheat differs from the cereal crops. Countries like Japan, Russia, Ukraine, USA, Poland, Canada, and Belarus have been scientifically working on buckwheat breeding before IBRA’s establishment. The first crop improvement program is done in common buckwheat (*Fagopyrum esculentum)* which started from 1901 till 1974 in Russia and Japan resulted in development of some popular and renowned varieties like Bogatyr (1901 to 1909), International Buckwheat Research Association (IBRA) was establish in September 1980 in Ljubljana, Slovenia which frequently arrange International buckwheat meeting to encourage and boost in international connection between scientist and their investigation, improving and producing new cultivars of buckwheat and allowing easy trade of genetical material among the member country and scientists. Due to the unique nature of crop cycle, development to achieve buckwheat of higher-yielding capacity is very slow which consequence can be seen as substitution of large acreage of buckwheat by different crops throughout the world. Because of which most portion of the International Buckwheat Research Association (IBRA) actions are solely focus on genetic improvement (Kreft,1983; Ohnishi, 1990; Ruszkowski, 1990).

3.4.2 Present State

After more than centuries research mostly focused on common buckwheat (*Fagopyrum esculentum*) currently other species of buckwheat mostly Tartary buckwheat (*Fagopyrum tartaricum)* and *Fagopyrum homotropicum* have been in growing interest due to easy post-harvest process, nutritional value and self-compatibility character respectively. In recent years Canada has become the leading country for the breeding of buckwheat. The development in variety of buckwheat results in production of higher groats yield and bigger seed size. There is increase in amount of tartary buckwheat (*Fagopyrum tartaricum)* production in recent year throughout the world due to its high ability to tolerate environment stress and self pollinating properties.

Latest Progresses in Overcoming Breeding Obstruction in Buckwheat

In vitro fertilization method is used in major species of Fagopyrum recently for inter-specific hybridization (Hirose et al. 1994; Lee et al. 1994 Campbell 1995). Despite all these progress this is not ideal for practical purpose. Cross association among *Fagopyrum esculentum* and *Fagopyrum homotropicum* was done for duplicating of the S locus to understand the heterostylism molecular biology and elements of reproductive organs at sub-cellular level which provide valuable character for breeding of buckwheat in future (Kawasaki et al. 2003).

3.4.3 Future state

For centuries buckwheat is an crucial grain and forage crop cultivated by humans. Buckwheat is short seasonal crop which don't required much of attention and care as they can do well in acidic and low fertile soil. They are very good in areas where rainfall is very low due to their branched root system with a primary taproot. Buckwheat is a highly nutritious whole grain that is considered as a super-food. They have well balanced necessary amino acids and rutin composition. Moreover, they don't contain gluten which is good for people related with gluten disorder. Apart from good qualities of buckwheat their production world wide has significantly decreased because of limitations like imperfect reproductive organ, self incompatibility and seeding failure in the post-zygotic stage. But progress in recent study and research has tried to examine the genetic traits and biotechnology to reduce the obstacles in buckwheat breeding (S.-H.Woo et al .,2016)

In vitro fertilization method is Common buckwheat is mostly cultivated in Europe, the USA, Canada, Brazil, South Africa, and Australia.Whereas Tartary buckwheat is cultivated in mountainous regions of southwest China, Central and East Europe In northern India, Bhutan, and Nepal (Lin, Tao, & Li et., 1992). There are some dates which support buckwheat have originated from China and it was planted as early as 5,000 to 6,000 years ago. In Nepal, it is also considered as a poor man's crop or alternative crops.

3.5 Types and Morphology

.The genus Fagopyrum comprises of about 15 to 16 species of plants,only two varieties Common buckwheat *(Fagopyrum esculentum)* and Tartary buckwheat (F*agopyrum tartaricum)* are used as food or feed whereas wild variety found as tetraploid, is sporadically utilized as cattle fodder or green vegetable. Buckwheat is usually cultivated for its unique black or gray, trilateral seeds with many uses mostly being a commodity and medicinal purpose. Both of them have similar uses as of cereal but do not belong to Poaceae (grass family) so that they are called pseudo-cereal. The buckwheat is an open-pollinated crop due to heterostylic self-incompatibility, though it has longer flowering period and produce many flowers, only few portion grow into trigonal dark hulled achenes (Halbrecq et al. 2005). Most of the buckwheat types and cultivars have indeterminate growth as main stem contains extremely changeable number of metamers I.e 5 to 30 , every offering one leaf and axillary bud per nod (Cawoy et al. 2009).

Fagopyrum esculentum **:**

Description:

Fagopyrum is thin erect annual herb with 3 - 5 ft height with a single main stem along-with branches.The cardinal stem is long, narrowed hollow, juicy and soft aside from nodes. The stem and branches varies from green to red color before maturity and eventually become reddish brown color at maturity. Leaves are wide triagonal and pointed tip with the length of 2 to 8 cm long and leaves are single at each node and born along the stem alternately.Inflorescence are occurred terminally and auxiliary, flowers of *Fagopyrum esculentum* are white to pink, perfect but incomplete due to lack of petals but instead they have calyx which is made of 5 petal like sepals and are commonly of white, pink or dark pink color with diameter of 6mm, pedicel length of 2-3mm, perianths of length 3mm, yellow color 8 nectaries are alternate to stamens, heterostyly and capitate stigma.

Achene is triangular with sides concave shaped longer than 5mm, brown to black soft hull, when mature seed size shrinks a little and turn its color from pale green to reddish brown. This species of buckwheat have many landraces and cultivars which can be differentiate by their seed shape example: seed with wings on angle and seeds without angle.

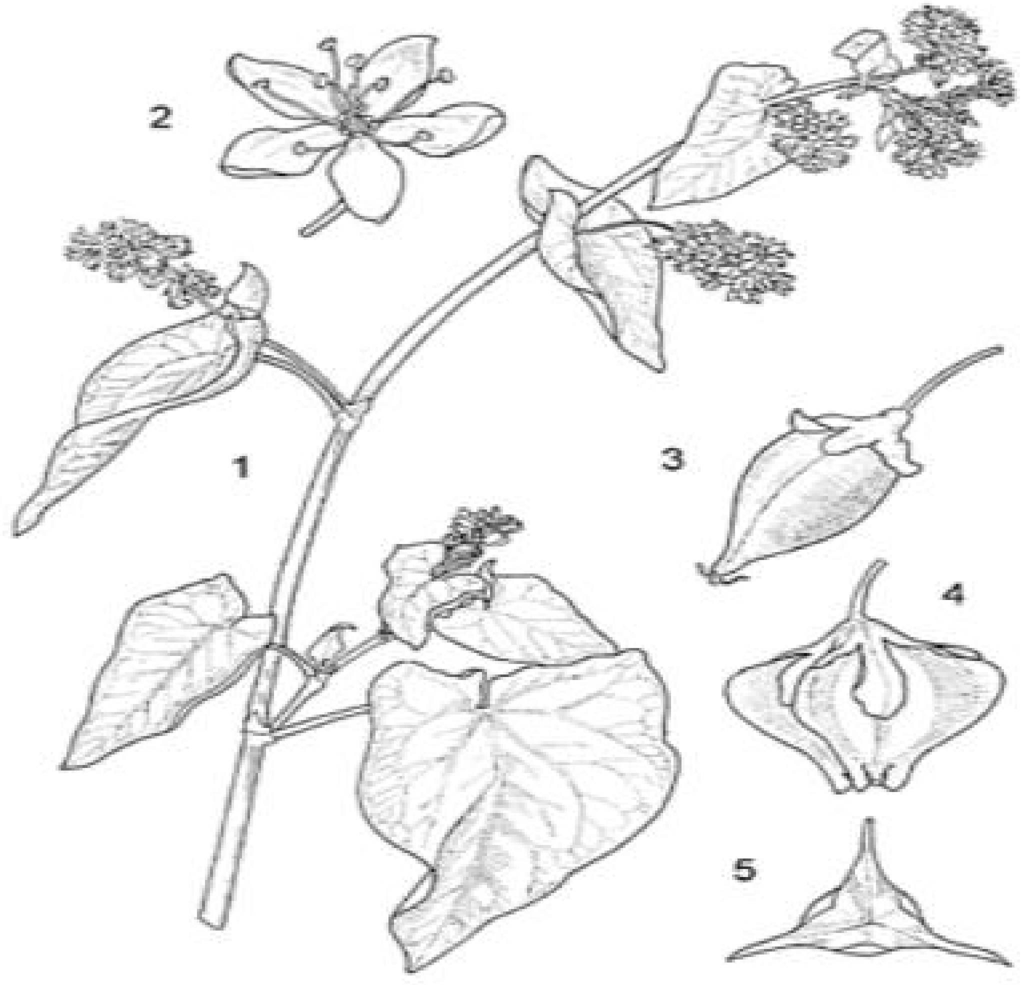

Fig. 2, 1.flowering branch, 2. Flower, 3. fruit without wing, 4. winged fruit, 5. winged fruit top view.

Source of Figure: https://uses.plantnet-project.org/en/Fagopyrum_esculentum_(PROTA)

***Fagopyrum tataricum*:**

Tartary buckwheat is an annual herb which grows up to 3- 5 feet tall depend on climate and location,branch or unbranched along the main stem which are striate with papillate on branch. Leaves are petiolate with triangular blades on both side of equal length, 2-8 cm. Leafs are cordate or hastate shape. Thick spicate or corymbose inflorescence.Flower are yellow - green color with diameter 2-5 mm, nonparticulate pedicles, perianth lenght 2 mm, yellow color 8 nectaries are alternate to stamens, homostyly and capitate stigma. Achenes are 5 mm in length and Triquetrous with rounded angles. Seed sizes and forms varies greatly example wings or spinous on angles. It is usually cultivated in the wintry alpine regions of Asia then any where else, due to its exceptional adaptability. This species can grow from altitude of 400 to 4400 m from sea level.

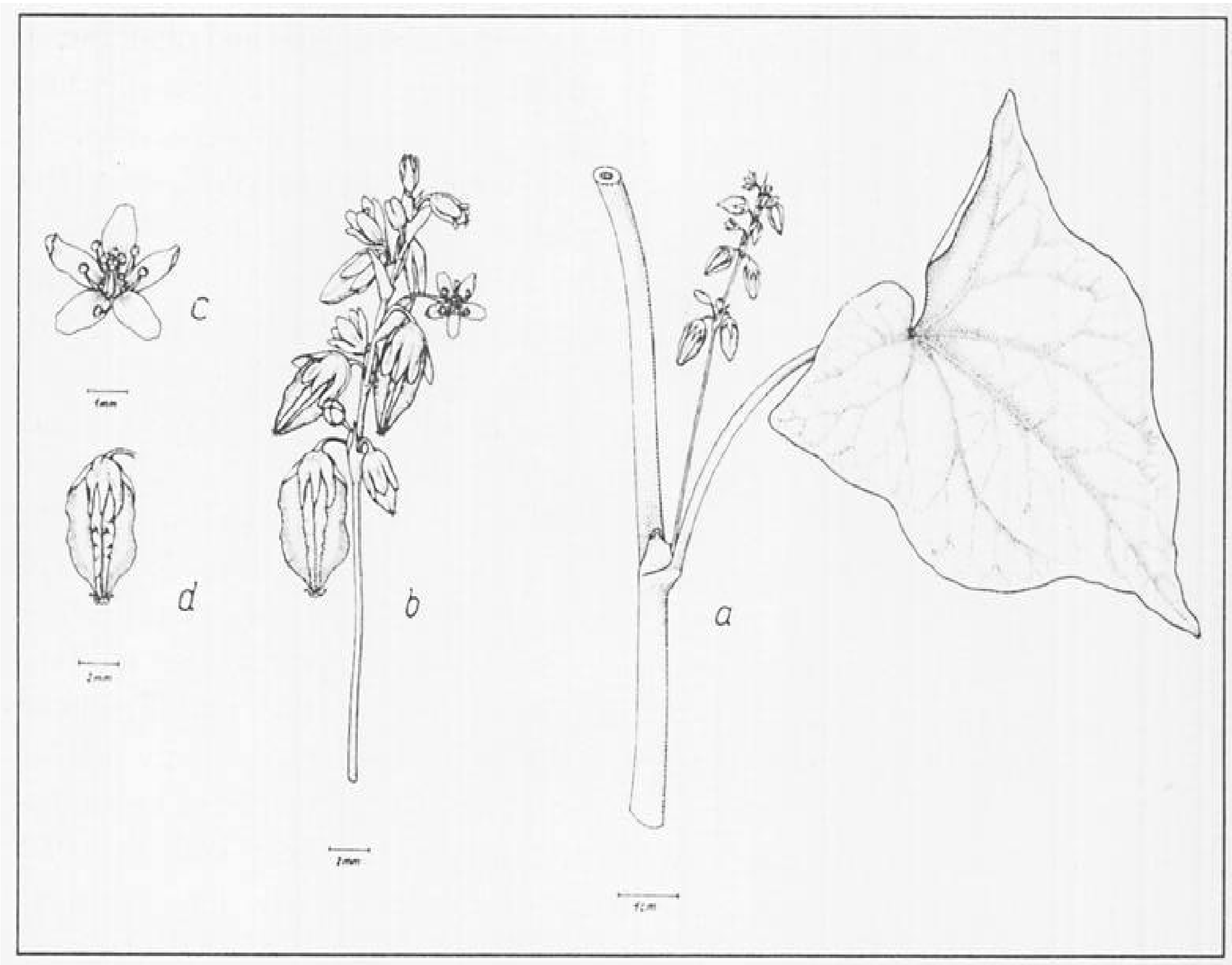

Fig. 3 *Fagopyrum tataricum* (Sketch by Ruth Kilian 1986), 1. Flowering branch, 2. Inflorescence with fruits, 3. Flower, 4. Fruit

Traits that separate *Fagopyrum tataricum* from *Fagopyrum esculentum* are the plants are mostly husky, leaves are more arrow shaped with more branches, flowers are rather small with indistinguishable greenish- white sepal which are not appealing for insects. Flowers are homomorphics, fertile and self-pollinating with out opening flower called cleistogamy.

3.6 Reproductive and botanical information:

Self incompatible species of buckwheat uses other medium for the cross- fertilization as they cannot fertilize themselves.

By insects: Insects are plays crucial role in cross- pollination of flowers. Flowers secret nectar from the glands located at the bottom of the ovary which attract insects. Morning and early midday are the only time glands secreting nectar, leaf-cutter bee were introduced and achieve decent level of pollination in buckwheat flowers (Cooke and Dalgleish, 1969). In another study at University of Pennsylvania, buckwheat grown in cage plot has the same seedset result as with the introduction of bees and larger insects (Hartly 1964).

By wind (anemophily) : Wind is also an important part for inter-fertilization of flowers. (Marshall 1994) report that seedset per plants is greatly influence by the wind source from plant, I;e plant close to wind source set more seeds than the plant away from wind source.

Fertilization: Buckwheat fertility has been reported quite below (Guan and Adachi 1992). This could be due to conflicting report quoted by many scientist, on the time taken for fertilization after pollination example, (Steven. 1912) 18 hours after, (Mahony. 1935) 48-60 hours after pollination for fertilization. On the other hand, (Adachi 1992) noted that fertilization of flower after pollination is dependent to the variety of crop and season.

3.7 Uses

Buckwheat is mostly cultivated as human commodity and as animal feeds. Due to its versatile use it is use as a vegetable,as a manure plant, as source a honey production, as a smoother plant to hinder weed growth etc.

Human consumption

Buckwheat have high nutritional values, it contains high amount of dietary fibers, proteins, vitamins, polyunsaturated fatty acids (PFA), minerals,antioxidants such as quercetin and rutin. Application of buckwheat in recent year has been increased due to its benefits on human health. Common buckwheat (*fagopyrum esculentum)* and tartary buckwheat (*fagopyrum* *tataricum*) are grown in different parts of the world and is used in various food formulation and production. Buckwheat is used in production of numerous local products relating to diverse culture and traditions. Buckwheat has multi use in Japanese and Korean culture, it is consumed mainly as noodles in Japan (soba) and Korea (nayengmyeon, makguksu), as a tea Japan (soba cha) and Korea (memil cha), as a distilled beverage buckwheat sochu in both countries. Roasted groats and flour are cooked in broth and use to make porridge and soup especially in Russia and Poland. In Italy buckwheat flour is use for making pasta, noodles and pizzoccheri mostly. In North America and Europe buckwheat flour is mixed with wheat flour to make biscuits, snacks, pancakes, pasta and cereal products. Likewise in the hilly region of southeast Asia buckwheat is major source of food. Various types of products are made from it like chapattis an unevenly leveled bread, pakora fried crisp, dhindo flour boiled in water etc. It is also used during religious festival and fasting. Whereas in china buckwheat is used in manufacturing of vinegar.

Smoother crop

Weed is a common problem in the world and only effective way to suppress and control weed without any negative impact on environment is using cover crop method. Buckwheat is perfect crop for weed control. Thick seedling rate along with its quick development and dense canopy it blocks light reaching the soil and hinder growth of slow developing weed. To maximize the weed suppression potential planting date should be timely I.e planting right before slow emerging weeds. (Jensen and Helgeson 1957) mentioned the benefits of buckwheat for management of many weeds like creeping jenny, leafy spurge, perennial pepper grass, sow thistle, knap weed and Canada thistle. Latest research have demonstrated that use of buckwheat residues seen effective against suppression of various weed species germination which including redroot pigweed; certain vegetables, including peas can also be inhibited, but only when planted into fresh residue. Buckwheat is documented to absorb excessive phosphorous and release through its fine roots which improve soil health and makes soil fertile for growth of the following crops. In addition, buckwheat flower draws many helpful insects.

3.8 Buckwheat composition and nutritional benefits

Buckwheat is a common commodity extensively used throughout the world. Buckwheat is composed of nutritionally valuable elements in very high amount and have many property of a functional food. For many years the production of buckwheat was declined, but since last few decades there has been a resurgence of interest in its cultivation because buckwheat content significant level of protein, starch, dietary fibre, some minerals, vitamins, flavonoids and bio-active components. A primary group of natural antioxidant in buckwheat is flavonoid, present in high quantity (Krkoskova et al. 2005).

It has anti-oxidant, anti-inflammatory and anti-hypertensive effects; due to this purpose it is considered as healthy food. It is rich in various nutrient such as protein, resistant starch, dietary fibers, Polyunsaturated fatty acids (PUFA) such as linoleic acid, flavonoids, Vitamin B1, B2 and C (Bonafaccia et al., 2003; Kitabayashi et al. 1995).

Whereas, the chemical constituent of buckwheat starch is similar to those in corn. Li and Zhang, (2001) has documented the flavonoid content and composition in buckwheat varies widely in two buckwheat species; they found tartary buckwheat contain 40 mg/g which is higher than in common buckwheat which contain 10 mg/g of flavonoids.

Buckwheat grains are important source of macro-elements such as K, Na, Ca, Mg (Wei et al. 2003) and micro-elements such as Zn, Na, Ca, Mg (Stibilj et al., 2004). Regarding fatty acid profile buckwheat contain 80% of unsaturated fatty acid with 40% of polyunsaturated fatty acid (PUFA)(Krkošková et al, 2005). Moreover, buckwheat grains are rich in total dietary fibre (TDF) and soluble dietary fibre (SDF); which are helpful to prevention of obesity and diabetes (Brennan 2005).

Some nutrient component like rutin, qucerient, and other flavonoids which cannot be synthesized by humans are also found in buckwheat (Kreft, Fabjan, & Yasumoto et al. 2006). Oomah & Mazza et al.,1996, reported rutin cannot be found in other cereals and pseudocereals but it is present in buckwheat seeds. On the other hand, the presence of flavonoids in buckwheat could be increased by germination (Kim, Kim, & Park et al. 2004). In conclusion, the digestibility of some large molecule nutrients (protein, starch, etc) in buckwheat, is relatively low. But if they were fragmented by enzymes in germination, their biological utilization rate should be improved greatly.

Protein: Buckwheat is remarkable source of amino acid in plant food stuffs. Buckwheat contain significant amount of albumin and globulin but low amount of prolamin and glutelin (Ikeda et al., 1991; Ikeda & Asami, 2000). In comparison, with other cereals, buckwheat seeds are high and have balanced amino acid content (Pomeranz & Robbins et al. 1972).

Unfortunately, the digestibility of buckwheat's protein in the human body is relatively low possibly due to tannins, phytic acid, and protease inhibitors (Ikeda & Kishida, 1993). The protein content has been reported to range from 12 to 18.9%. Hull contain low amount of protein, about 4%, however in embryo it reaches 55.9% (Pomeranz & Sachs, 1972). The protein content of buckwheat flour varies from 8.5% to 19%; which depends on the variety, pesticides used and fertilization (Fornal 1999).

Polysaccharides and dietary fibre: Starch is the major storage components of buckwheat grains. The starch content varies from 59 to 70% of the dry mass; which is strongly influenced by climatic and cultivation conditions (Qian and Kuhn 1999). However the investigation which was done by Polish researchers has reported the presence of starch content in three different buckwheat cultivar lies in narrow range from 63 to 66% DM. (Stempińska and Soral-Śmietana 2006).

It contain a high level of resistance starch; which have several health benefits such as reduction in risk of colon cancer, hemorrhoids, diverticulosis, and constipation, increase in fecal bulking, and modulation of blood glucose and blood cholesterol levels (Skrabanja et al., 2001). Sharma et al. 2008 has reported that resistance starch has a prebiotic activities. The formation of starch in buckwheat grains vary from that of cereals starch.

Amylose present in buckwheat starch granules varies between 15-52% and the degree of polymerization fluctuate 12 to 45 glucose units (Campbell, 1997). Whereas, resistance starch content 33-38% of total starch of uncooked buckwheat, which can be reduced to 7-10% via cooking.

Lipids: It contain 1.5 -4% lipid (Steadman et al ., 2001), whereas the content of raw fat in buckwheat flour exceeds 3% (Soral-Smietana 1987). Maximum concentration of lipids in buckwheat was found in the embryo (7-14%), and the minimum in the hull (0.4-0.9%) (Bonaffacia et al., 2003). Triacylglycerides are the main components of lipid containing fatty acids from C12 to C22, and the main fatty acids are oleic (42%), linoleic (32%), and palmatic acid (16%) (Soral-Smietana 1987).

Vitamins and minerals:

Li and Zhang 2001, has reported the mineral content in buckwheat grains (dry base) reaches: 2-2.5% in whole grains, 1.8-2% in kernel, 2.2-3.5% in dehulled grains, 0.9% in flour and 3.4-4.2% in hull. Buckwheat are rich in potassium, calcium, magnesium, and sodium. Buckwheat is also important source of microelement such as manganese, zinc, and iron (Wei et al. 1995). Trace element such as chromium and selenium are occasionally detected in low levels (Stibilj et al. 2004). Buckwheat grains also contain vitamins: B1, B2 and B6 (Fabjan et al. 2003).

Buckwheat production

Within the past decade, the cultivated land has doubled from a million to over two million hectares due to its excellent nutritional values (Popovic et al. 2013a, 2013b).The world production of buckwheat in the year 2017 was approximately 2.83 million tonnes, which was led by Russia with around 1.52 million tonnes and China with 1.45 million tonnes. Which was followed by Ukraine, France and Kazakhstan with 180, 127 and 120 thousand tonnes respectively (FAOSTAT, 2017).

3.9 Influence of different physiological and stress conditions on Buckwheat

Ecological factors such as water, light, temperature and pH values play an important role in seed germination (Rizzardi et al. 2009). Mature seeds are typically low in moisture and nearly deactivated metabolically which assures their long-term survival during storage and in the unfavorable field condition (Morad Shaban 2013). Germination process is a life line that ensures the survival of all kinds of plant. Changes in environmental factors greatly impact on the overall performance of plant. Plant are exposed to different types of environmental and other stresses during the period of their life cycle. Due to breeding of crops with the purpose of high production and strengthening so as to cultivate them beyond its ecological periphery which make them more susceptible to different types of stress in crops (McKersie and Leshem 1994).

3.9.1 Water relation with seed viability

Water is one of the most essential factor which decides seedling survival (Elberse and Bremen 1990). Water absorption is first signal for germination which helps to hydrolyze constituent like protein, starches, triglycerides, phytic acids salts present in seed which are exhibit at insoluble form during storage condition. In order to germinate, metabolic activities like activation of enzyme, combination and breakdown, synthesis of various component must occurs. This operation needs large amount of energy as it supplies dynamic force for cell development which is derived from starch breakdown into glucose (Koller and Hadas 1982). Every species of seed have its particular critical moisture value for germination to occur. In absence of critical moisture value metabolic activities for hydrolysis, synthesis and the manufacturing of energy is nearly absent or inactive. Water stress inflicted serious limitations in germination of seeds (Bonvisutto and Busso, 2007). Germination percentage and germination speed of seed decrease during lower availability of water or water stress (Scott et al. 1984). Different types of dormancy and internal mechanism are restricted at water stress condition (T.W. Hegarty. 1978). Stress interfare differently with different stage of development and functioning, low germination percentage and speed during germination (Scott et al. 1984), effects the structural development and functioning of growing tissues resulting in small size leaf during vegetation period (Schurr et al. 2000), reduce fertility in new flower and impact on seed set at later stage (Slawinska and Obendorf. 2001).

Due to global warming, climate change is seen world wide with it extreme event occurrence is more frequent, in future the drought occurrence will probably rise beyond arid and semi-arid territory (Albritton and Meira Filho .2001, Chaves et al. 2002). Water stress is likely to occur more often in rainfed areas which heavily effect development in plant (C. Delperee, J.M. Kinet, S. Lutts. 2003).

3.9.2 Temperature relation with seed viability

Temperature shows a considerable function in determining the important factors of germination process like germination percentage, germination speed and germination time. Temperature plays significant role in water absorption, which is very important for starting life phenomenon in seed. Germination process initiates when sufficient level of humidity and warmth are meet, which plays a principal role to start chemical reactions in seed to provide it with life(Esan. 1960, Grzesiuk and Kulka. 1981). Seedlings that grows early will oppress those seeds that develop at after stage so early development of seedling is vital (Birhuizen. 1974). Buckwheat plant is thermophile in nature and cultivated in spring season. It is very difficult to determine exact value for minimum, maximum and optimal temperature for growth due to controversial statement claimed my many authors. Various author documented dissimilar temperature supporting germination and growths. Primarily three temperature range support germination of seeds, namely

Minimum temperature; temperature where seed begins to germination and below which germination stops, Observation according to (Hryncewicz 1992) is 7-8 C, while by (Grzesiuk and Kulka 1981) is 3-5 C.

Maximum temperature; temperature above which seed stops up taking water causing seedling to wither eventually leading it to death. Observation according to (Grzesiuk and Kulka 1981) is 35-45 C, by (Ruszkowaka 1981) is 31 C.

Optimum temperature; a temperature at which growth and development of seeds is seen. Observation according to (Platek and Oles 2000, Ruszkowska 1981) is 25-26 C, while by (Grzesiuk and Kulka 1981) is 25-31 C and by (Hryncewicz 1992) is 20-25 C.

Prijic, L., Jovanovic, M. and Glamoclija, D. (1998) documented from their experiment on soyabean that, due to high temperature and water stress during seed filing result in high number of green and wrinkled seeds. Further testing of these green and wrinkle seed for its vigor showed low performance than normal seeds.

3.9.3 Residual chlorophyll content in seed relation with viability of seed

Chlorophyll is a green pigment created by plants, cyanobacteria and algae which help absorb some wavelength of light and convert them into energy called photo-system which are necessary for plant function and development. Photosystem is very effective during green leafs development (Ohashi et al. 1989).On the contrary, chlorophyll present in old leaves degrade which help to transfer nitrogen and carbon to newly form organ (Hortensteiner. 2006).

In a similar pattern as leaves, chlorophyll formation and degradation also occurs in developing seed (Saori Nakajima et al. 2012). (Ward et al. 1995) recorded that breed and environment influence chlorophyll break down at the later stage of seed maturation which affect final level of chlorophyll in seed. Chlorophyll content in seed produced under suitable atmospheric conditions may have low to no chlorophyll. Harvesting properly mature seed has higher germination rate and have better storable seeds than seed harvested before mature stage (Sanhewe and Ellis. 1996a, 1996b). Seed setting is one of the important phase during development of seed, any unfavorable ecological stress during this point result in accumulation of chlorophyll in seed and bad quality seed. Chlorophyll aggregation in seeds block the activity of abscisic acid (ABA) by presenting antibody against abscisic acid which is responsible for many function in development of seed (Philips et al, 1997).

Saori Nakajima et al. (2012) did research on relation of between seed maturation, chlorophyll, storability and documented chlorophyll content on seed depend on the maturity of seed, seed with high chlorophyll affect the organelles development in germinal cells which result in production of low quality seeds. Chlorophyll content in seeds affect seed storability as germination rate quickly decline during storage, from Saori Nakajima et al. (2012) experiment it is shown that seed with high chlorophyll fail to germinate after 23 month of storage whereas normal seed germinate 75% after 42 months which indicates that chlorophyll content in seeds affects seed germination rate and longevity.

4. Materials and Methods

4.1 Methods

Entire variety of seeds used for the experiment were gathered from the Crop Research Institute, Prague, Czech Republic. This study was carried out by testing twelve Varieties of buckwheat they are as follows 1.Kis Cebelica, 2.Zamira 3.Hrussowta 4.Billy, 5.Zita, 6.Kora, 7.Bamby, 8. Kis Eva, 9.Zoe, 10.Kis Doris, 11.Panda, 12.Harpe. Before sampling all seed were mixed in their respective bags because lighter seeds tend to settle on top than heavier ones. For sample seeds of suitable size are chosen to represent the sample lot. To minimize changes in seed moisture, the sampling exercise was done quickly.

Establishment of germination tests

I. **Standard germination test (G_{st})** was established in the laboratory, following International Rules for Seed Testing by the International Seed Testing Association (ISTA). Seed lots were placed in germination containers that were put in the seed germination chamber. In each germination container, 3 plane filter papers (Hahnemühle) were placed at the bottom and a single pleated filter paper on top. Seed lots were prepared in two replications per 100 seeds. Seed lots were subjected to two incubation temperatures of 20 °C and 15 °C (set in the germination chamber)at a stable temperature controlled with the precision of ±1ºC. Whole germination process is carried out without the source of light.and two moisture levels; higher dose 30 ml (standard/optimal) and lower dose 20 ml (stressed variant) of tap water.

II. **Residual chlorophyll evaluated seed germination test** was performed in laboratory where six cultivars of buckwheat were randomly selected from twelve from twelve cultivars used for standard germination test. We measured chlorophyll and separate them according to the amount of chlorophyll content I:e High chlorophyll content and low chlorophyll content and perform germination test with 30 ml volume of water under different temperature (15C and 20C).

Evaluation of germination and cotyledons emergence

Seeds with primary root and cotyledons were evaluated as normal seedlings from day 2 to day 6 after ‘planting’. Evaluation of germinates was done primarily by calculating germination percentages of two replications per 100 seeds for each sample. A germinated seed was identified as having roots and a visible coleoptile at least 3 mm long. Total germination was calculated as the sum of daily values (cumulative germination). Germinated seeds were removed using tweezers and counted every 24 hours until the end of the experiment. Non germinated seeds were left in the germination container, counted and returned to the germination chamber. Abnormal seeds were counted and removed using tweezers. After every two day’s evaluation, the germination containers were returned to the germination chamber for counting cumulative germination after every 48 hours.

At day 6, all germinated and abnormal seeds were counted and removed. Any seeds that had germinated, i.e. radicle visible, but not achieved the 4x seed length rule were to be classified as ‘low vigour’. Ungerminated seeds must be cut tested and classified into either ‘empty’ or ‘filled non germinated’. An ‘abnormal’ seedling is any seedling that falls into the abnormal category according to Section 5.2.8 of ISTA (2015) rules. cotyledons emergence was measured on day 5 and day 6,

The following germination variables were measured for germination:

Total Germination is an indicator of the basic characteristics of viable seeds that are capable of further development from germination, subsequent embryo growth to the formation of an adult plant. This is the percentage of germinated seeds out of the total seed rate.

Clean and dry box are used where paper towel is laid at first and 50-50 seeds in either side are placed with gap in between adjacent seed to avoid clustering of seed once they started germinate and facilitate in further purpose such as counting, separation of individual. Two replication were separated by empty ridge space and 2 black dots.

4.2 Materials and sampling

Experiment is divided into two groups

I. 9600 seeds were incubated in germination chamber from twelve cultivars for the test

Seed lots were prepared in two replication for every 100 seeds and for test we use 800 seed from every sample(cultivar). For evaluation of seed germination under temperature (15C and 20C) and volume of water (20ml and 30ml)with four different arrangement.

II. 4800 buckwheat seed chlorophyll are measured from 6 samples(cultivar) in Mobile Chlorophyll Analyzer (MCA) and separate them according to their chlorophyll content and perform two different tests.

Germination test of high chlorophyll content seeds under different temperature (15C and 20C) with 30ml volume of water. 1 cultivar 8 replication with 400 seeds ,200 seeds in two different temperature.

Germination test of low chlorophyll content seeds under different temperature (15C and 20C) with 30ml volume of water.

Germinated seeds are counted at period of particular hours. To maintain the desired moisture level inside the seed germination chamber few empty sample boxes are filled with water. Seeds with minimum 3 mm length of sprouts are considered as germinated.

4.3 Statistical analysis

The germination testing for seed vigor data were evaluated using SAS statistical program, version 9.4 (SAS Institute. Cary, USA) and the Analysis of variance(ANOVA) was determined. For further evaluation the Tukey Multiple Comparison (HSD) method at a significance level of $P \leq 0.05$ method was also used.

5. Results

5.1 Experiment using iXeed chlorophyll analyzer and Seed germination chamber

Our experiment showed a very significant influence of the chlorophyll for all the evaluated parameters (GE2, GE4, GE, CoT2). Similarly, Cultivar also displayed very significant difference for all the experimented traits. The effect of temperature on the various traits was mostly very significant and significant, apart from the GE. Highest influence was observed on the GE2 and CoT2 under influence of temperature. The impact of combinations Ch + Cu was very significant to entire parameters. Whereas, Ch + Cu + T were mostly very significant, apart from GE4; on the other hand, Ch + T and T + Cu were very significant on GE2, CoT2 and insignificant on GE4, GE (Table 1).

Table 1. The effect of chlorophyll on the germination test of buckwheat under various parameters such as temperature, cultivar and their various combinations (ANOVA Fishers *F* value)

	2nd day germination (GE2)	4th day germination (GE4)	Germination energy (GE)	6th day cotyledon germination (CoT2)
Chlorophyll (Ch)	154.97**	157.12**	134.32**	96.08**
Cultivar (Cu)	37.01**	49.59**	26.01**	16.05**
Temperature (T)	12110.0**	5.31*	1.90	6013.20**
Ch + Cu	3.96**	7.32**	10.79**	5.32**
Ch + T	154.97**	0.07	0.10	68.05**
T + Cu	37.01**	1.01	0.90	13.92**
Ch + Cu + T	3.96**	0.62	3.48**	5.33**

P<0.05* - significant; p<0.01** - very significant, non- significant component are not illustrated.

5.1.1 The effect of different chlorophyll content in germination test of various components

In this section, we illustrate only chlorophyll effects on overall seed performance under various parameters. There were very significant influence of different chlorophyll content on all analyzed components (GE2, GE4, GE and CoT2), and the very considerable difference was observed in GE4 $F(1, 95)= 157.12$ at $P<0.0001$ and least in CoT2 $F(1, 95)= 96.08$ at $p<0.0001$ within same group.

Nevertheless, GE4 $F(1, 95)= 157.12$ and GE $F(1, 95)= 134.32$ had the highest significant difference among whole groups and their interactions at $P<0.0001$.

Interactions of Ch + Cu had very considerable difference for all evaluated parameters, similarly, Ch + Cu + T was very significantly influenced in most of the traits, apart from the GE4 (Table 1).

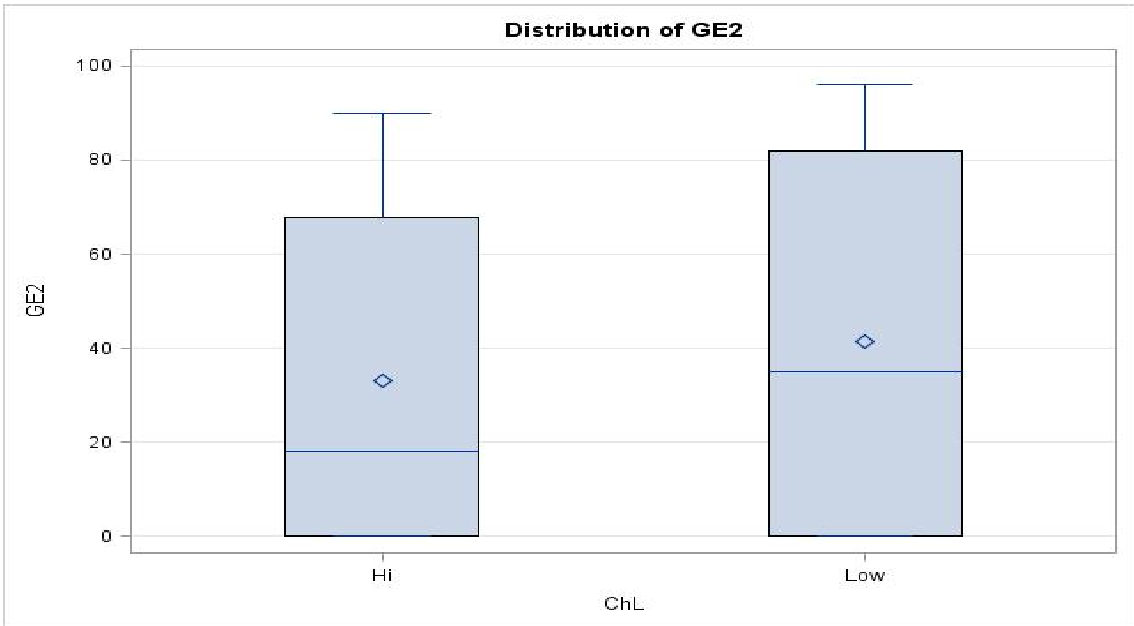

Figure 4. Comparison of germination percentage in GE2 of different chlorophyll content

Chlorophyll remaining inside seed has considerable influence in seed germination. Overall germination in second day germination count (GE2) was higher in low chlorophyll content seeds than in high chlorophyll content seeds (Fig 4).

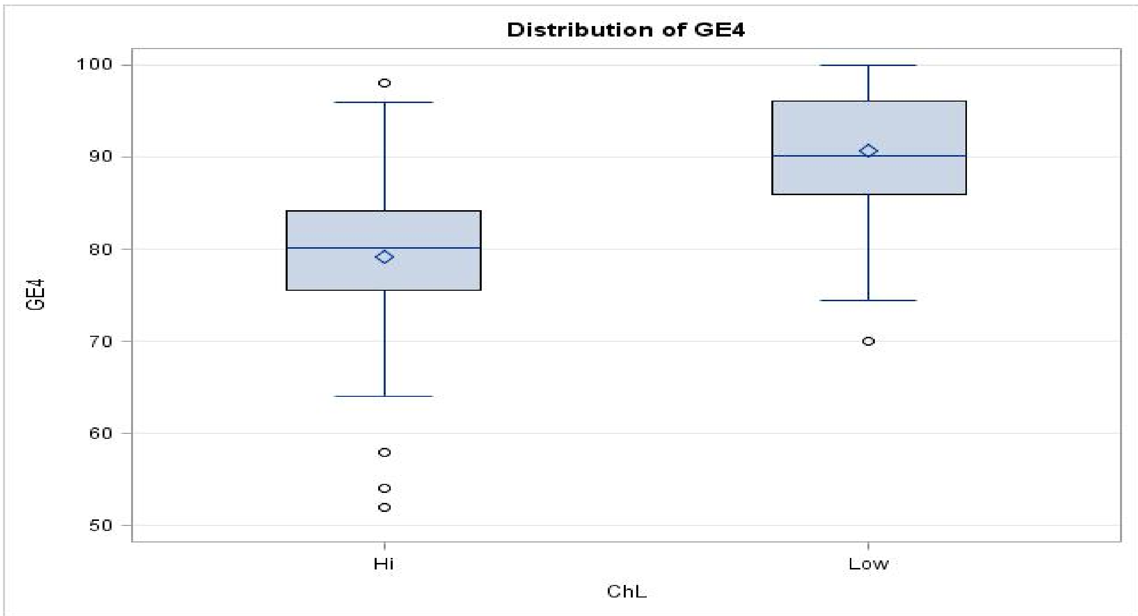

Figure 5. Comparison of germination percentage in GE4 of different chlorophyll content

There is a very significant difference in germination between high chlorophyll seeds and low chlorophyll seeds in fourth day germination count (GE4). Figure 5 clearly illustrates, low chlorophyll seed germination percentage is higher than low chlorophyll seeds.

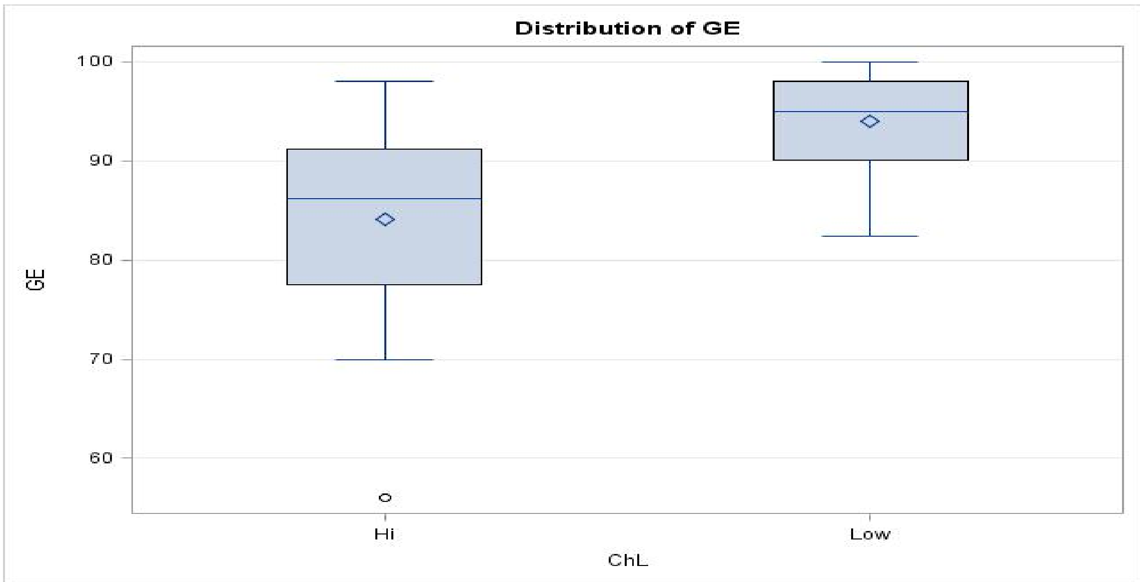

Figure 6. Comparison of total germination percentage of different chlorophyll content

Total germination percentage is considerably greater in low residual chlorophyll content seeds than high residual chlorophyll content seeds (Fig 6).

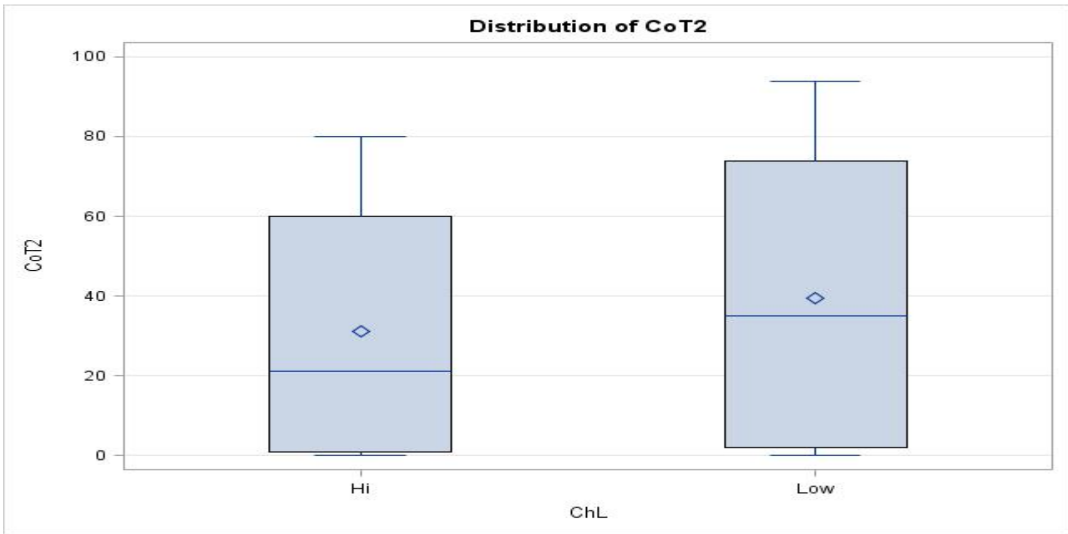

Figure 7. Comparison of cotyledon emergence of different chlorophyll content

Similarly, cotyledon emergence from seeds was observed more from low chlorophyll seeds than from seeds with high chlorophyll content (Fig 7).

Table 2. Mean number of germination test under chlorophyll influence. Number with same alphabets are not significantly different - Tukey's HSD test at the significant level of P<0.05

Germination evaluating factors	Chlorophyll content	Tukey grouping	Mean
2nd day germination (GE2)	High	B	33.05
	Low	A	41.49
4th day germination (GE4)	High	B	79.13
	Low	A	90.72
6th day Cotyledon emergence (CoT2)	High	B	31.08
	Low	A	39.5
Total germination (GE)	High	B	84.05
	Low	A	93.97

The Tukeys' post hos test reveled difference in chlorophyll content had very significant effect on the seed vigor. Seed with low chlorophyll content had more positive germination test result than seeds with high chlorophyll (Fig5)(Fig6)(Fig8). Germination energy was highest in low chlorophyll seed than high chlorophyll seeds (Fig.7). Summary of germination test on different traits within and among different parameters under chlorophyll influence is provided in Table 2.

5.1.2 The effect of temperature difference in seed germination test on various parameters

The experiment shows, there was very significant difference in temperature for early germination (GE2) of seeds $F(1, 95)= 12110$, $p<0.0001$ (Fig. 1) and Cotyledon emergence (CoT2) $F(1, 95)= 6013.2$, $p<0.0001$ (Fig. 2). GE2 and CoT2 was mostly influenced by temperature than by other factors such as chlorophyll and cultivars. There was significant difference in germination count in 4th day $F(1, 95)= 5.31$, $p<0.05$ (Fig. 3). The influence of interactions Ch+T and T+Cu were very significant in GE2 and CoT2 whereas Ch+T+Cu was very significant in all components,apart from GE4 (Table 1).

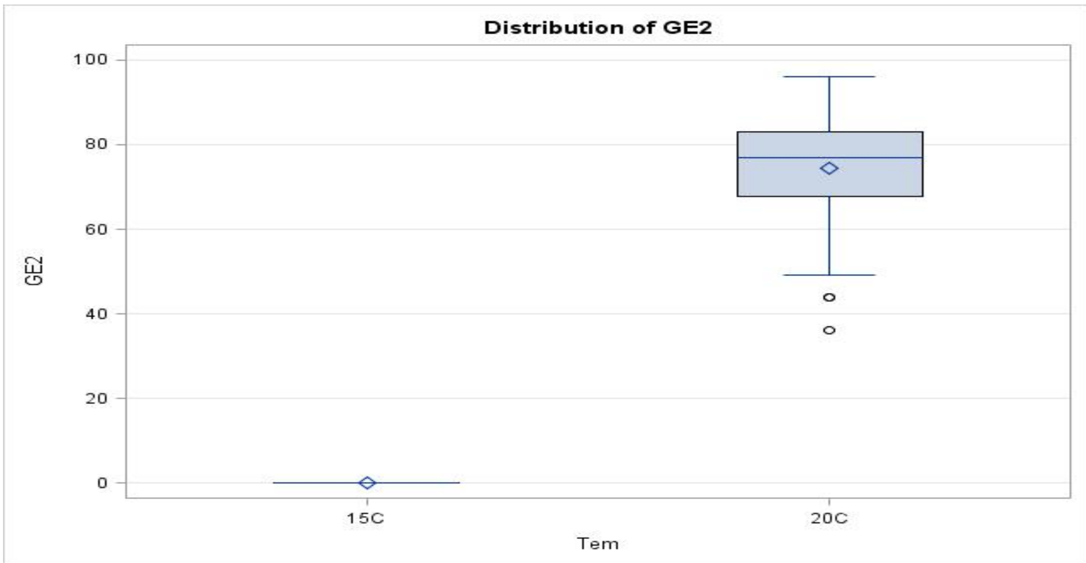

Figure 8. Comparison of germinated seed count on second day in two different temperatures

Among all the testing conditions, temperature was the most significantly influential for the early germination of seeds. Figure 9 demonstrates the difference between seed germination in different temperature, where 74.04% seed are germinated in 20C and 0% germination in 15C.

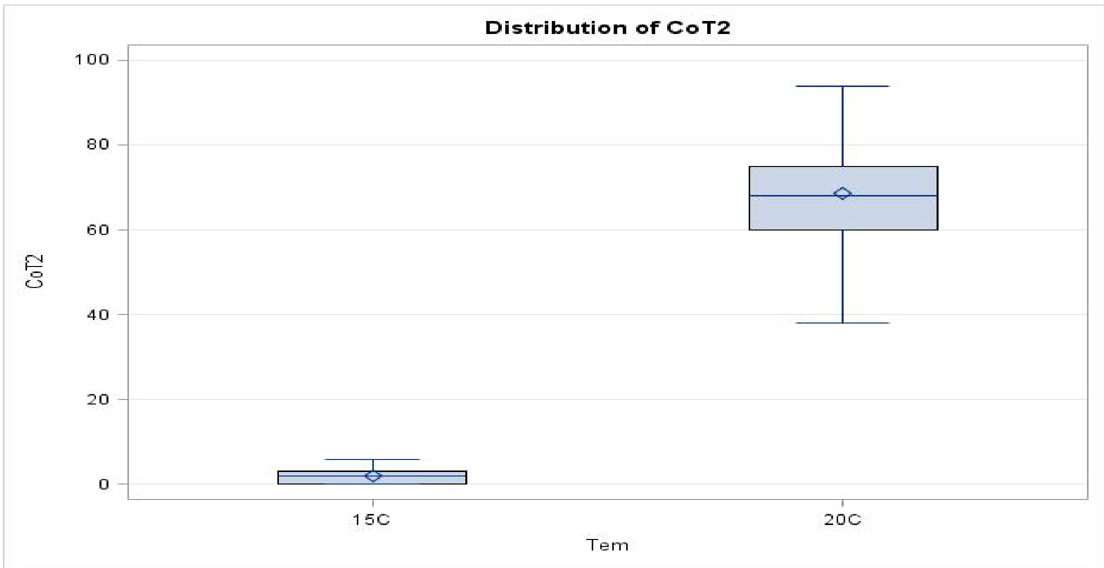

Figure 9. Cotyledon emergence count in sixth day in two different temperatures

Cotyledon emergence was second highest, very significantly affected by the temperature. Percentage of cotyledon emergence is higher in 20C than in 15C with value of 68.5 and 2 respectively (Fig 10).

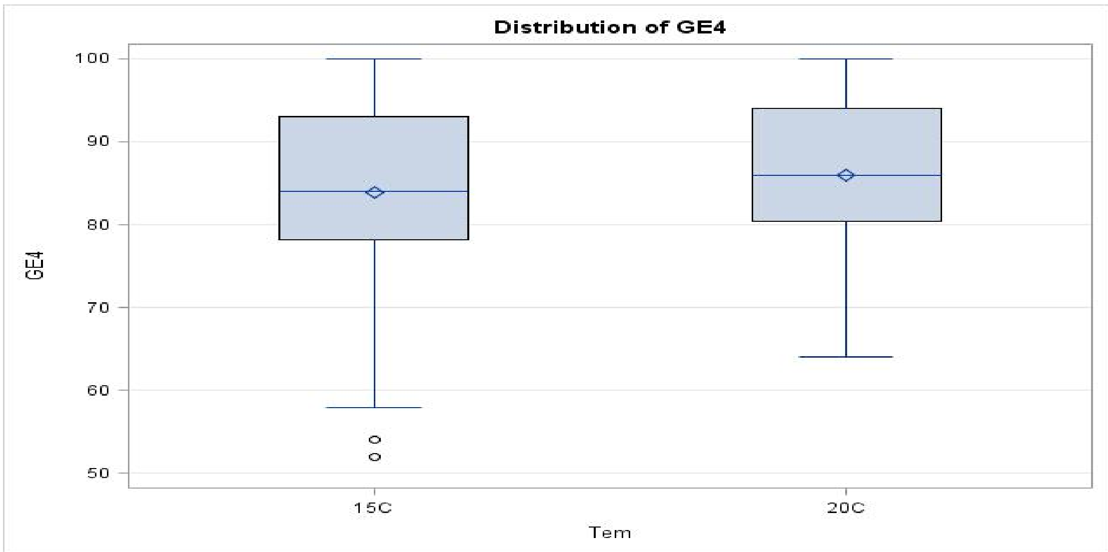

Fig 10. Comparison of germinated seed count on fourth day in two different temperature

There was significant difference in germination by temperature where, germination was slightly higher in 20C than in 15C (Fig 10).

According to Tukeys post hoc test, there was very significantly different in temperature, which influence the early germination (GE2) and cotyledon emergence (CoT2) more than other two factor (chlorophyll and cultivar) and their interactions. Whereas, germination count on 4th day (GE4) was significantly different between different temperature where 20C had slightly higher germination than 15C. While there was no significant difference by temperature on germination energy of seed. The maximum value of GE2 was 74.04 in 20C and the lowest value of CoT2 was 2 in 15C. Detail analysis of mean value for germination test under temperature influence is given in Table 3.

Table 3. Mean number of germination test under temperature influence. Number with same alphabets are not significantly different - Tukey's HSD test at the significant level of P<0.05

Germination evaluating factors	Temp.	Tukey grouping	Mean
2nd day germination (GE2)	20C	A	74.04
	15C	B	0
4th day germination (GE4)	20C	A	85.9927
	15C	B	83.8615
6th day Cotyledon emergence (CoT2)	20C	A	68.58
	15C	B	2
Total germination (GE)	20C	A	89.6
	15C	A	88.42

5.1.3 The effect of cultivars on the germination test over various component

A very significant difference of the cultivar was seen on all analyzed components (GE2, GE4, GE and CoT2), where GE4 $F(5, 95) = 49.59$ at $p<0.0001$ showed greater number of significant difference than other with in same group. Followed by GE2 $F(5, 95) = 37.01$ at $p<0.0001$, GE $F(5, 95) = 26.01$ at $p<0.0001$ and CoT2 $F(5, 95) = 16.05$ at $p<0.0001$. Similarly, there was very significant influence viewed in all evaluated components in the interaction of Ch + Cu, While Ch + Cu + T was mostly very significant except GE4 which was insignificant. On the other hand, response of Cu + T was significant on GE2 and CoT2 (Table 1).

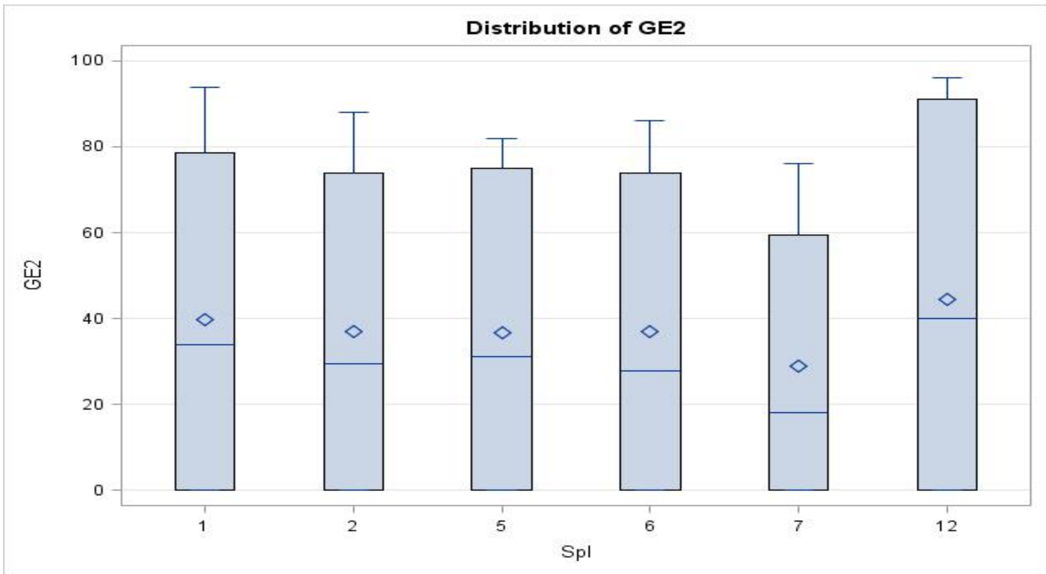

Figure 11. Comparison of germinated seed count in second day within cultivars

Cultivar number 12 performed better than among the tested cultivars followed by cultivar number 1. On the other hand, cultivar 7 performed lowest in second day germination tests (Fig11).

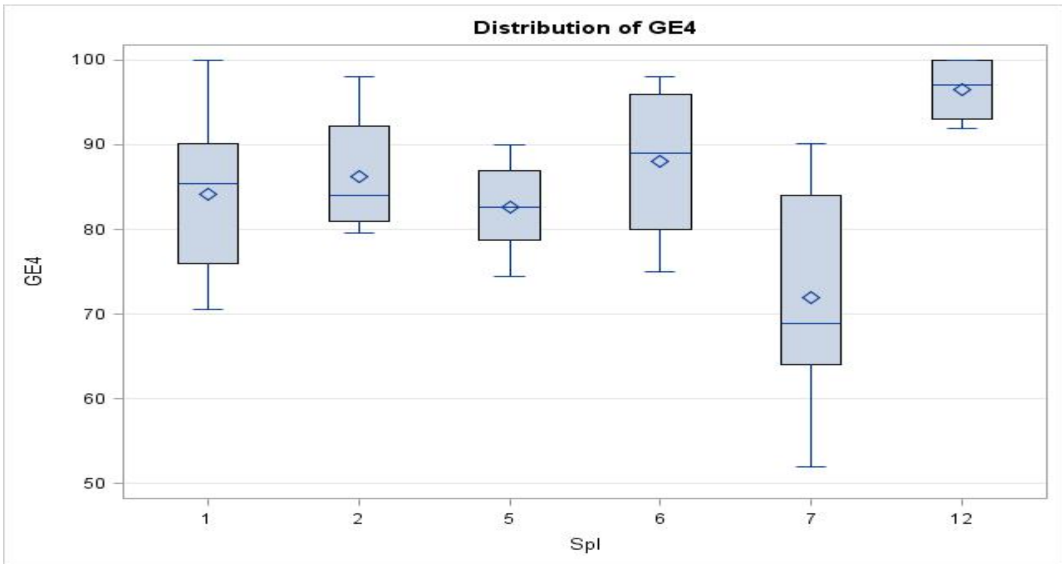

Figure 12. Comparison of germinated seed count in fourth day within cultivars

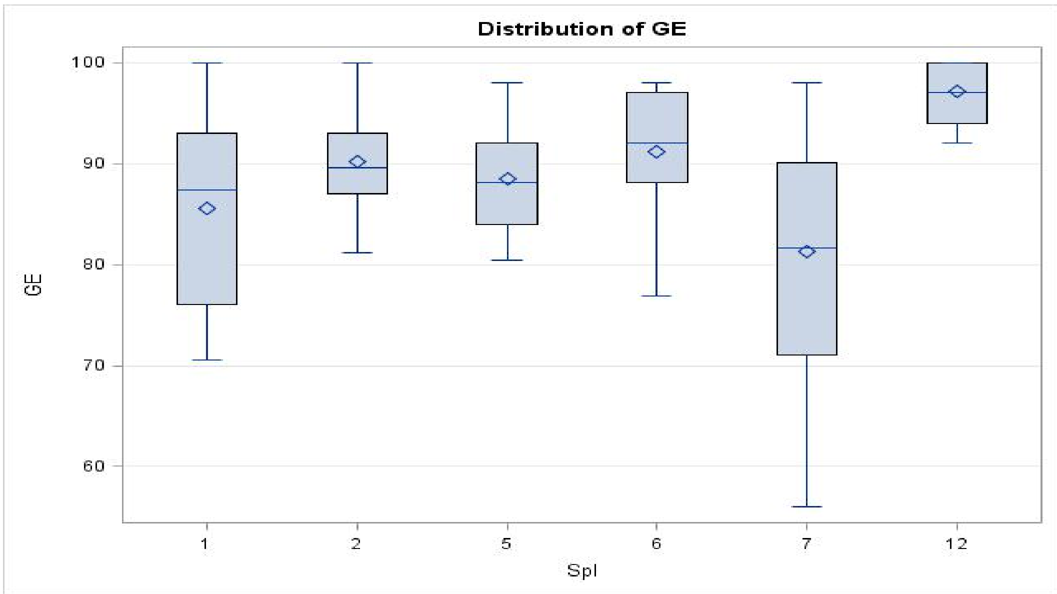

Figure 13. Comparison of total germination of cultivars

We observed similar pattern in GE4 an GE during test where we found cultivar 12 had the highest germination value of 96.51% and 97.13% respectively. Contrary to that cultivar 7 had the lowest germination score in both GE4 72.01% and GE 81.34% (Fig 12, 13).

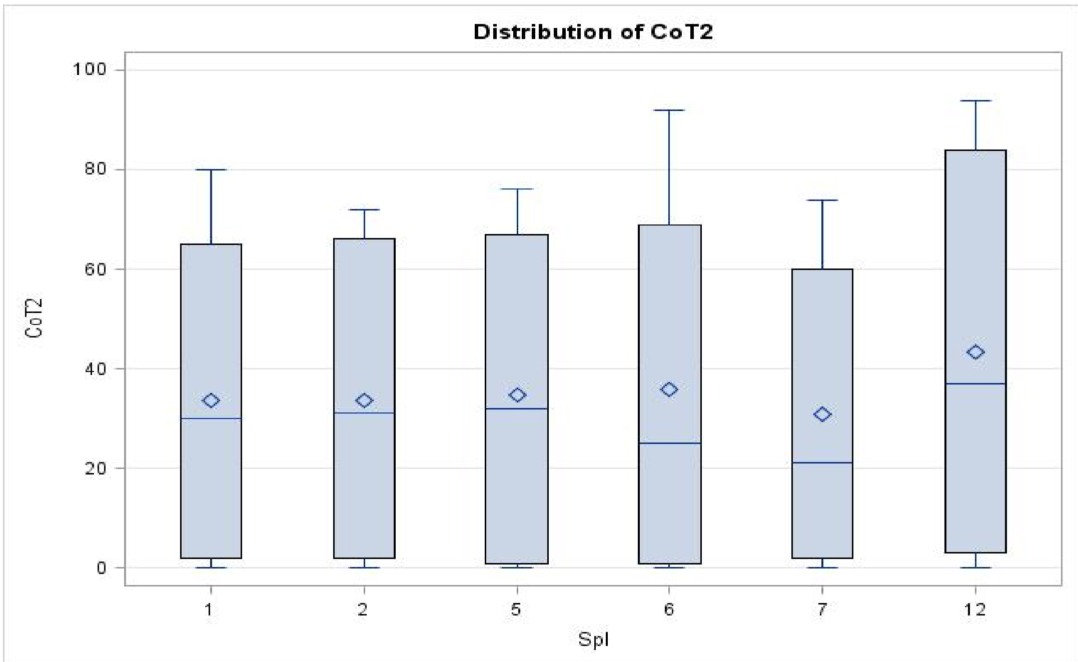

Figure 14. Cotyledon emergence count in sixth day within cultivars

Our result showed that cotyledon emergence was highest in cultivar 12 (43.25%) and lowest in cultivar 7 (30.75%).

The results from Tukey tests describe the statistical significance between mean value of cultivar.

Sample 12 displayed high seed vigor in all tested parameters, with maximum value in GE 97% and lowest in CoT2 43.25%.

On the other hand, sample 7 perform very poor in all the tested parameters, with maximum value in GE 81.34% and lowest in CoT2 28.98%.

Moreover, sample 1, 2, 5, 6 perform average in most of the tested parameters, (Table3)

Table 4. Mean value of seed germination by the cultivars. Number with same alphabets are not significantly different - Tukey's HSD test at the significant level of P<0.05

Cultivars number	GE2	GE4	GE	CoT2
1	39.62^{b}	84.13^{bc}	85.62^{cd}	33.63^{bc}
2	37.01^{b}	86.26^{bc}	90.22^{b}	33.63^{bc}
5	36.67^{b}	82.65^{c}	88.53^{bc}	34.75^{bc}
6	36.84^{b}	88.01^{b}	91.21^{b}	35.75^{b}
7	28.98^{c}	72.01^{d}	81.34^{d}	30.75^{c}
12	44.51^{a}	96.51^{a}	97.13^{a}	43.25^{a0}

5.2 Experiment using seed germination chamber for standard germination

(Table 5) Germination test under various parameters such as temperature, cultivar, Water volume and their various combination (ANOVA Fishers *F* value).

	2nd day germination(GE2)	4th day germination (GE4)	Germination energy (GE)	6th day cotyledon germination (CoT2)
Cultivar (Cu)	269.65**	761.93**	505.86**	232.42**
Water (W)	8.63**	2.70	0.47	10.96**
Temperature (T)	7629.13**	84.10**	1.93	15801.7**
Cu + W	17.07**	4.63**	2.68**	3.46**
Cu + T	178.18**	15.56**	9.52**	195.65**
T + W	34.68**	2.17	6.61*	63.11**
W + Cu + T	12.81**	2.76**	1.80	3.65**

P<0.05* - significant; p<0.01** - very significant, non- significant component are not illustrated.

Our study for germination test demonstrates a high significant difference of the cultivars to whole analyzed factors (GE2, GE4, GE, CoT2).

likewise, temperature was very significantly influenced except in GE. Temperature showed the highest significant difference in CoT2 throughout the test.

While on the contrary, water was reported very significant in GE2 and CoT2 and insignificant on other two parameters.

Interactions between Cu + W and Cu + T was detected very significantly influenced throughout the entire tested factors. Similarly, T + W and W + Cu + T were mostly very significant apart from GE4 and GE respectively (Table 5)

5.2.1 The effect of temperature difference on germination test over several components

The experiment revels that temperature was highly influential for early germination GE2 $F(1, 191) = 7629.13$ at $p<0.0001$ (Fig 16) and cotyledon emergence CoT2 $F(1,191)=15801.7$ at $p<0.0001$(Fig 18). Even on the GE4, temperature have greatly influenced germination (Fig 17), whereas total germination (GE) of seed was not influenced by temperature (Table 5).

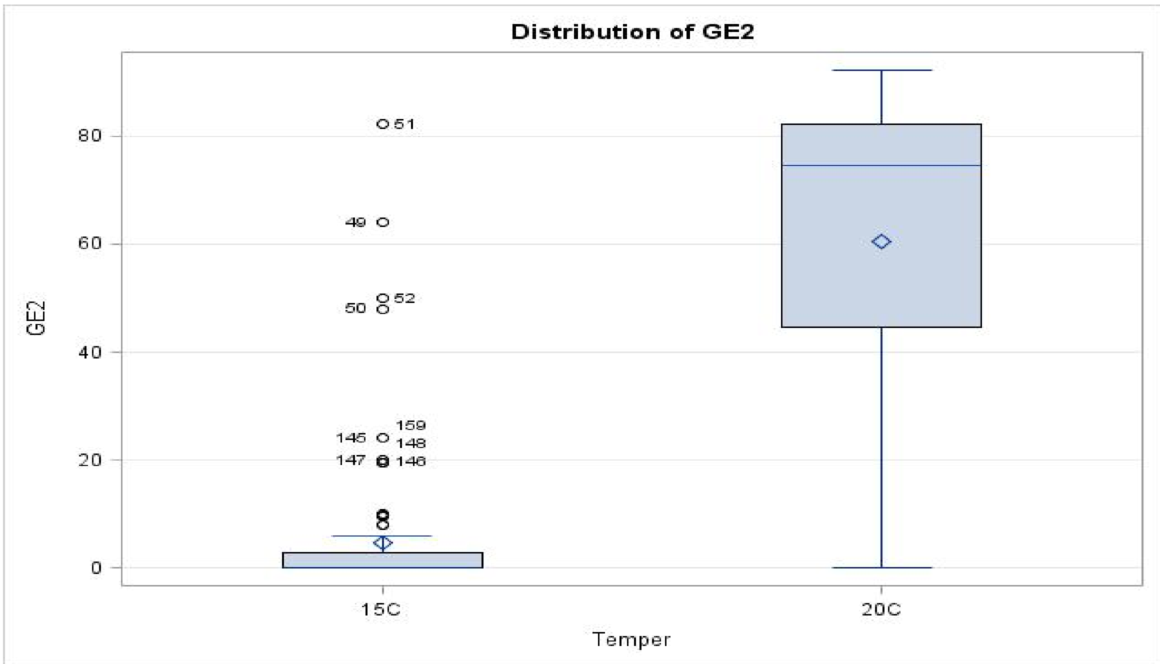

Figure 15. Comparison of GE2 in different temperature.

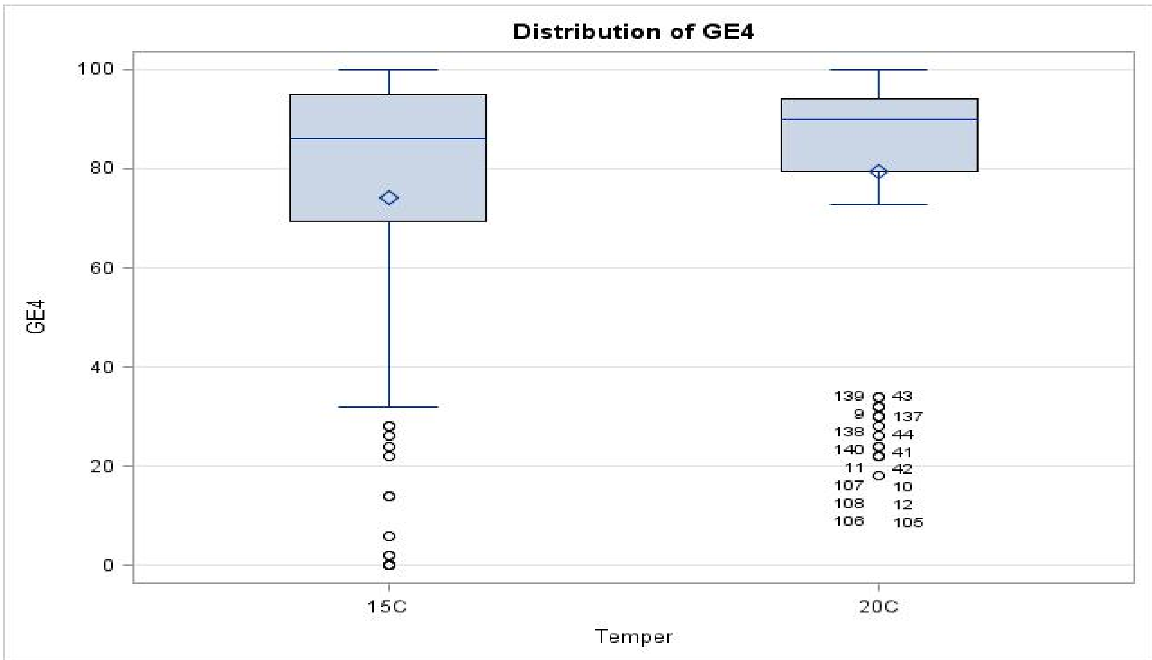

Figure 16. Comparison of GE4 in different temperature

Our standard germination test revels that there was a very significant difference in germination between different temperature. Second day germination count (GE2) showed, germination was substantially higher in 20C than in 15C (Fig 15). likewise, temperature influence was seen in fourth day germination count(GE4) also where germination was slightly higher in 20C than in 15C (Fig 16).

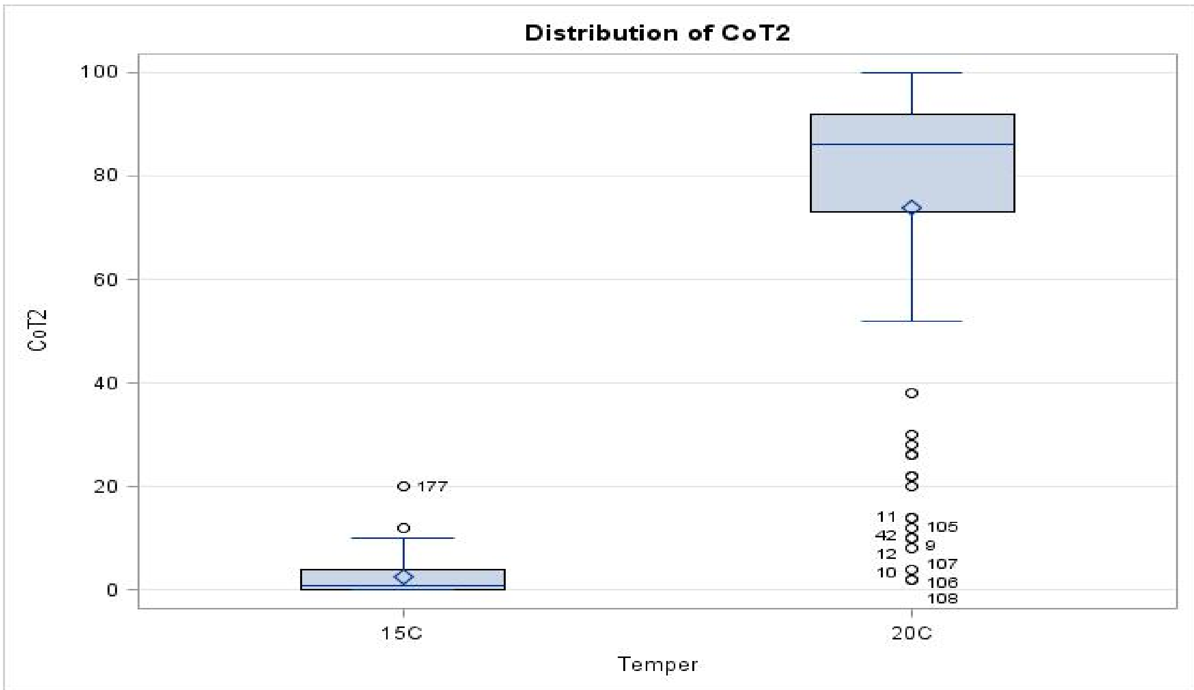

Figure 17. cotyledon emergence test on sixth day in different temperature

Cotyledon emergence was the most greatly influenced due to temperature. Cotyledon emerged seed counts were higher in 20C than in 15C (Fig 18).

Tukey seed germination test report revealed that early seed germination(GE2) and cotyledon emergence(CoT2) was much greater in 20C and less in 15C which was 60.49 and 4.73 for GE2(Fig .16) and 73.77 and 2.56 for CoT2(Fig 18) respectively. On GE4 also 20C seemed more effective for germination than 15C. While there was no significant difference in GE (Table 6).

Table 6. Evaluation of temperature effect on germination percentage of twelve different cultivar. Number with same alphabets are not significantly different - Tukey's HSD test at the significant level of P<0.05

Germination evaluating factors	Temperature	Tukey grouping	Mean
2nd day germination (GE2)	15C	B	4.73
	20C	A	60.49
4th day germination (GE4)	15C	B	74.22
	20C	A	79.55
6th day Cotyledon emergence (CoT2)	15C	B	2.56
	20C	A	73.77
Total germination (GE)	15C	A	81.14
	20C	A	82.14

5.2.2 Comparison of different water volume effect on the germination test over various traits.

The results showed a very considerable difference GE2 $F(1, 191)=8.63$ at $p<0.0001$ and in CoT2 $F(1, 191)=10.96$ at $p<0.0001$. On the other hand, GE4 and GE were not that effectively influential on the test.

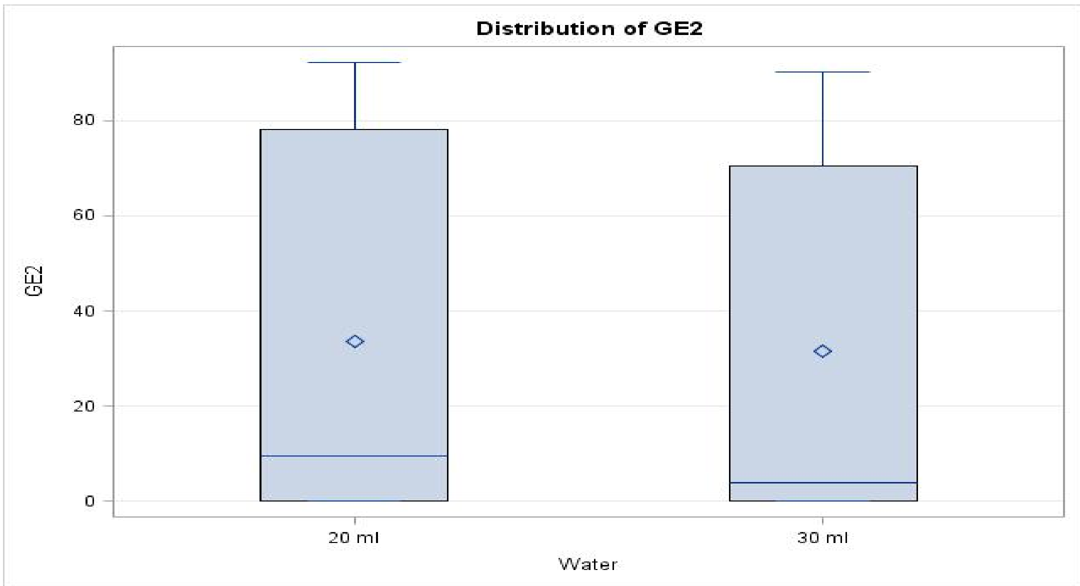

Figure 18. Comparison of GE2 in different volume of water.

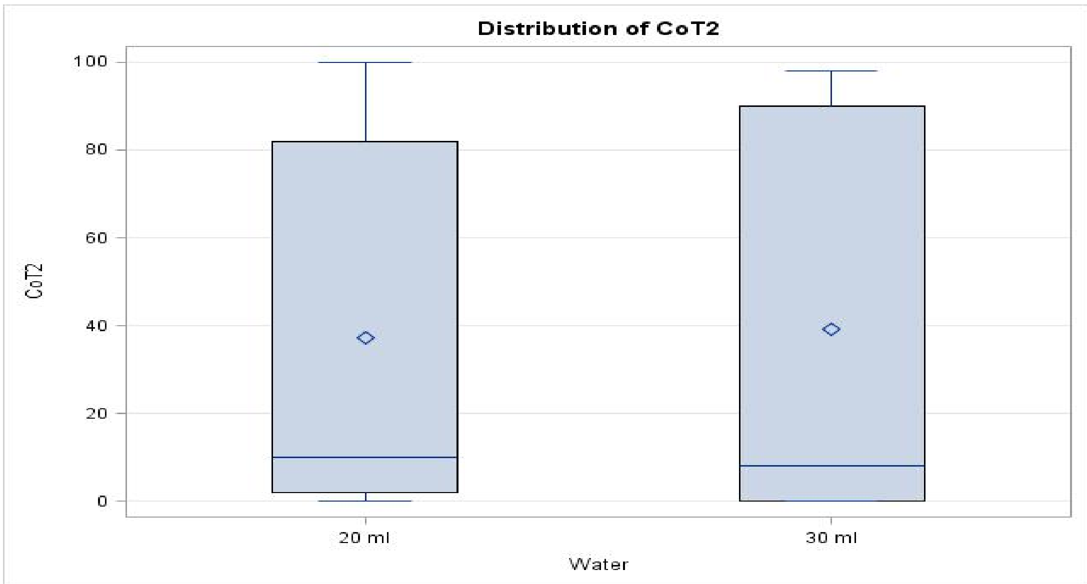

Figure 19. Comparison of cotyledon emergence in different volume of water

From the Tukey result we can observe that 20ml water seems to have more influence on the germination than with 30 ml. GE2 was 33.5 in 20ml water and 31.67 in 30ml water(Fig19). Surprisingly, CoT2 was less in 20ml water I;e 37.29 than 30ml water I;e 39.1 (Fig .20). While there is no any significant difference in mean value of GE4 and GE (Table 7).

Table 7. Evaluation of different water level influence on germination. Number with same alphabets are not significantly different - Tukey's HSD test at the significant level of $P<0.05$.

Germination evaluating factors	Temperature	Tukey grouping	Mean
2nd day germination (GE2)	20ml	A	33.5
	30ml	B	31.67
4th day germination (GE4)	20ml	A	77.36
	30ml	A	76.4
6th day Cotyledon emergence (CoT2)	20ml	A	37.29
	30ml	B	39.1
Total germination (GE)	20ml	A	81.93
	30ml	A	81.52

5.2.3 **Comparison of different cultivars performance on the germination test over various traits**

The study illustrates a very significant difference of the cultivars for the entire evaluated parameters. Greatest value of significant difference within cultivars was observed in GE4 $F(11, 191)=761.93$ at $p<0.0001$ and GE $F(11, 191)=202.86$ at $p<0.0001$. Cu + W and Cu + T combination was detected very significantly influenced by all the evaluated factors. Likewise, W + Cu + T were mostly very significant except on GE (Table 5).

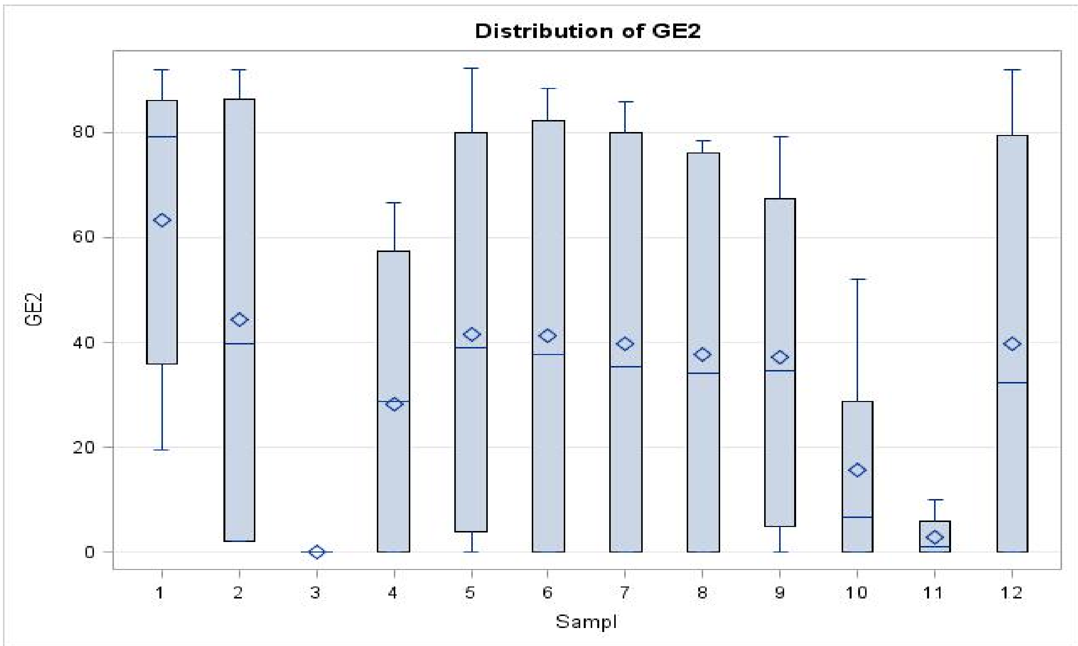

Figure 20. Comparison of germination test on GE2 of twelve cultivars

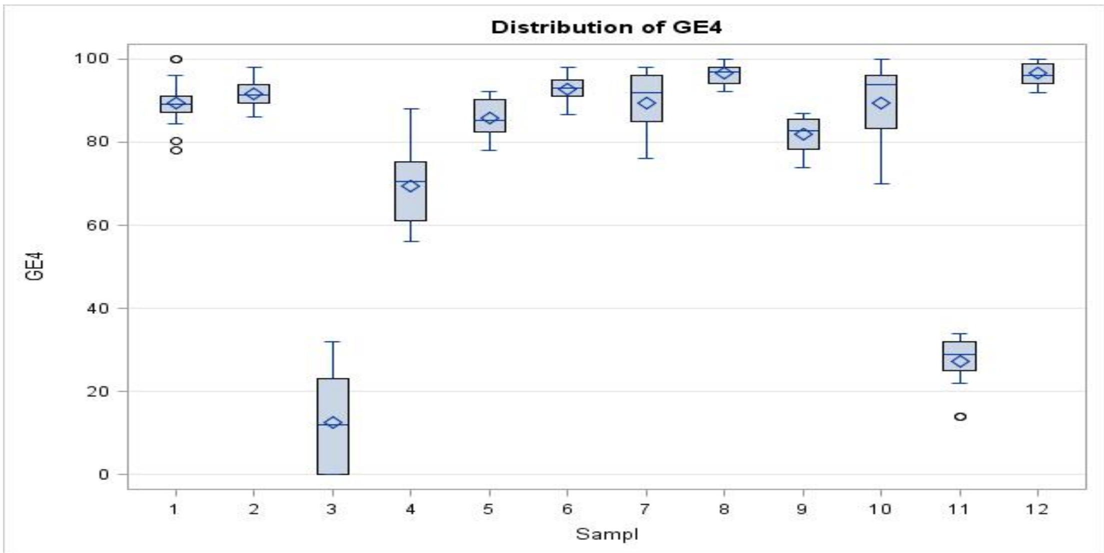

Figure 21. Comparison of germination test of twelve cultivar on GE4

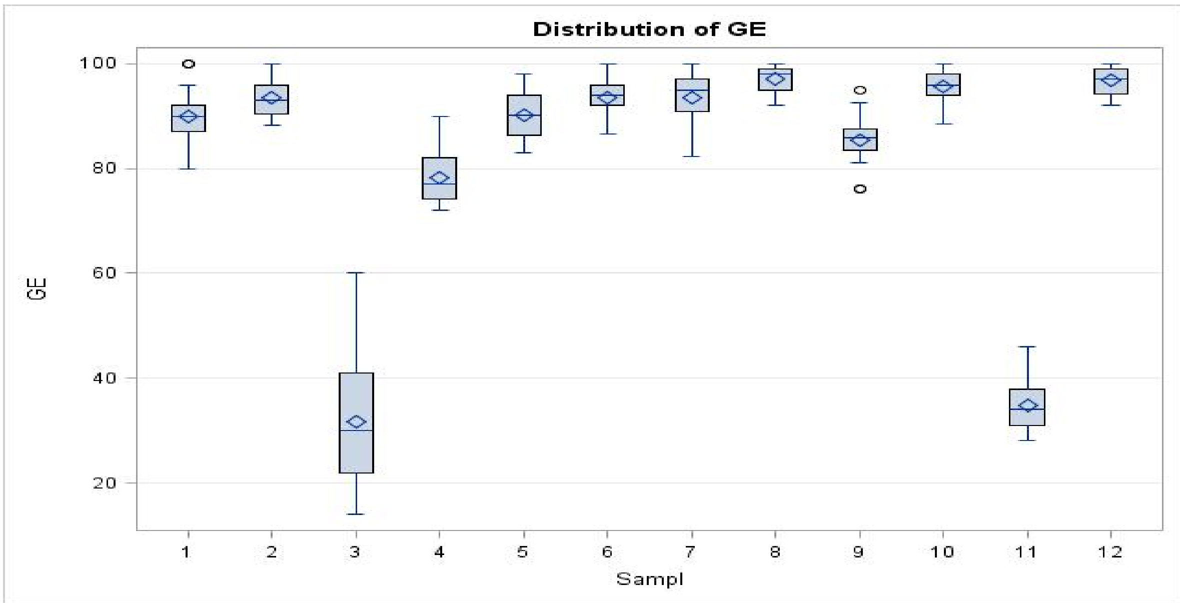

Figure 22. Comparison of total germination of twelve cultivars

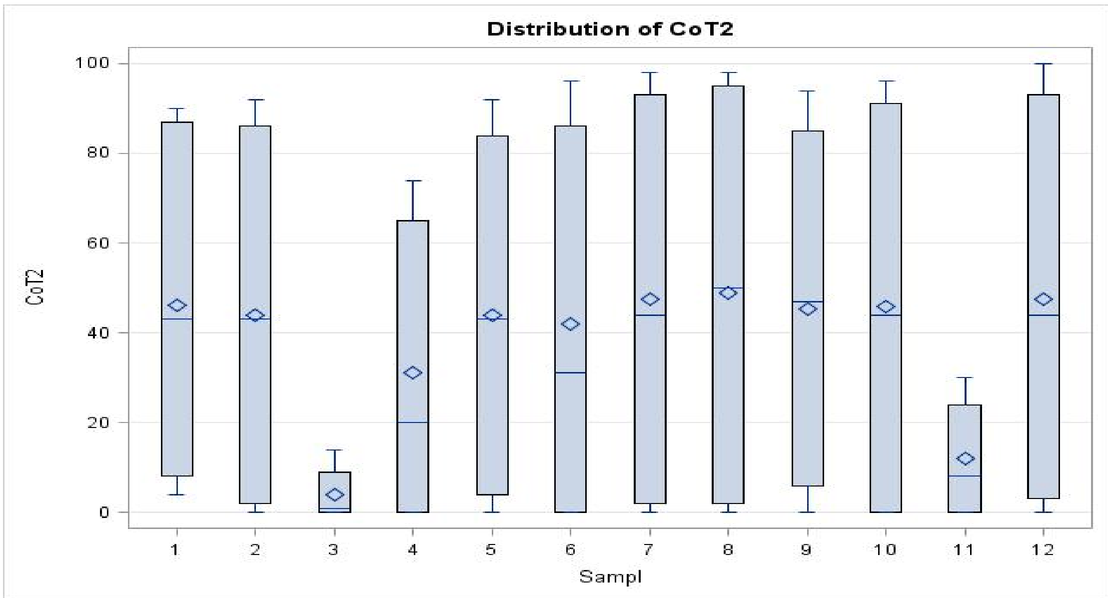

Figure 23. Comparison of cotyledon emergence of cultivars

Tukey result table revels sample 1, 2, 6 perform best on GE2 with value of 63.27, 44.37, 41.34 and contrary to this, sample 3, 10, 11 perform the least GE2 with mean value of 28.25, 15.62, 2,98 respectively.

Similarly, in GE4 sample 12, 8, 6 showed high performance with value of 96.54, 96.53, 92.87 and sample 11, 3, 4 displayed low performance with score of 27.25, 12.62, 69.33 respectively. Additionally, in GE sample 8, 12, 10 illustrated better performance with marks of 97.16, 96.79, 95.57 and sample 3, 4, 11 perform less with mean value of 31.59, 78.4, 34.87

respectively. Eventually, in CoT2 sample 8, 12, 7 demonstrated high cotyledon emergence with mean score of 48.88, 47.68, 47.38 and sample 3, 4, 11 was observed least performing 3.88, 31.13, 11.88 respectively.

Table 8. Evaluation of cultivars performance on germination under various traits. Number with same alphabets are not significantly different - Tukey's HSD test at the significant level of P<0.05

Cultivars with sample no.	GE2	GE4	GE	CoT2
1	63.27^{a}	89.34^{bc}	89.96^{bc}	46.13^{abc}
2	44.37^{b}	91.65^{b}	93.65^{bc}	44^{bc}
3	0^{f}	12.62^{g}	31.59^{e}	3.88^{f}
4	28.25^{d}	69.33^{e}	78.4^{d}	31.13^{d}
5	41.43^{bc}	85.83^{cd}	90.17^{bc}	44^{bc}
6	41.34^{bc}	92.87^{ab}	93.62^{ab}	42^{c}
7	39.69^{bc}	89.34^{bc}	93.65^{ab}	47.38^{ab}
8	37.62^{c}	96.53^{a}	97.16^{a}	48.88^{a}
9	37.09^{c}	81.81^{d}	85.34^{c}	45.38^{abc}
10	15.62^{e}	89.32^{bc}	95.57^{a}	45.75^{abc}
11	2.98^{f}	27.25^{f}	34.87^{c}	11.88^{e}
12	39.67^{bc}	96.54^{a}	96.79^{a}	47.63^{ab}

6. Discussion

The effect of chlorophyll

Ward et al (1995) found that residual chlorophyll amount in seed was highly influenced by the types of cultivar and atmosphere they grow. We found very significant influence of chlorophyll on all parameters. Saori Nakajima et al. (2012) classified the relation between chlorophyll, seed maturity and report that immature seed contains high level of chlorophyll leading to poor development of seed which result in low germination rate and poor crop stand. Our experimental result supports Saori Nakajima et al. (2012) as we observed high chlorophyll seeds performed very less in comparison to the seed performed with low chlorophyll level and also found many cases of anomalies in high chlorophyll content seeds in later stages. The table 1 result display a very significant ($p<0.0001$) difference of chlorophyll on all parameters. According to Saori Nakajima et al (2012) chlorophyll content in seed influence seed storability and with time of storage germination capacity of seeds drops.

Sanhewe and Ellis (1996a, 1996b) stated that seed germination percentage was higher and better storage life when chlorophyll content in seed was low, harvesting in proper maturity under suitable condition result in little to no residual chlorophyll in seed. Our experiment fully convinced with their statement as we observed that seed viability was greater in low chlorophyll content seed than in seed with high chlorophyll. On average, the value of GE2, GE4, GE, CoT2 are 33.05, 79.13, 84.05, 31.08 respectively in high chlorophyll content seed and 41.49, 90.72, 93.97,39.5 respectively in low chlorophyll content seed which clearly illustrates that low chlorophyll seed have high vigor(Table 2).

The effect of temperature

According to Esan. (1960), Grzesiuk and Kulka,.(1981) the effect of temperature was very crucial factor of germination process, as it creates suitable environment for proper functioning of various life giving activities inside seed. Different author reported various temperatures helping germination and growth, as different species has different temperature range of growth and development.

From our investigation we found that the early germination (GE2) at 20C are significantly higher with mean value of 74.54 than in 15C with mean value of 0 and similar result was seen in sixth day cotyledon count where number of cotyledon emerged seed count was higher in 20C with mean value 68.58 and 15C with mean value of 2, so our experimental result support

Hryncewicz (1992), where Hryncewicz (1992) stated that optimal temperature for the seed germination and development was 20C - 25C. Temperature below and above optimal temperature affected performance of seed and seedling activity.

The effect of water

According to Elberse and Bremen (1990) water is one the most important element for germination and proliferation of plant. Bonvisutto and Busso (2007) reported water stress severely affect the seed germination. Likewise, Scott et al. (1984) also observed speed of germination and germination percentage of seed reduced during water stress. We carried out our experiment with two different volume of water, 20ml and 30ml and observed insignificant difference in germination energy(GE) and germination count on fourth day (GE4)Table 5.

We found more number of germination in second day(GE2) at 20ml volume of water than 30ml. On the other hand, we observed more number of cotyledon emerged in 30ml volume of water than 20ml.

The effect of cultivars

Every species and variety of plant has their own competence level for growth and development. In our investigation we used twelve different variety of buckwheat and perform standard germination test and germination test under residual chlorophyll influence where we detected that cultivars of sample number 1 (Kis Cebelica), 2 (Zamira), 6 (Kora), 8 (Kis Eva), 10 (Kis Doris), 12 (Harpe) had very good result in all measured factors than other cultivars of buckwheat.

7. Conclusion

Our findings confirmed that presence of residual chlorophyll in buckwheat seed influence seed performance and its vigor. On our experiment of germination test we found that low residual chlorophyll content seed performance over all tested parameters were higher than high residual chlorophyll content seeds. In another words, seeds with low residual chlorophyll have high vigour.

We observed temperature effect on the germination speed, germination percentage and emergence of cotyledon.

We even find strange result from our different water volume test where germination is seen more in 20ml water and cotyledon emergence is seen higher in 30 ml water.

And lastly, from our individual cultivar performance on germination test, sample 12 (Harpe) perform excellent among other cultivars.

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9. List of Abbreviations

DNA = Deoxyribonucleic Acid

RAPD = Random Amplified Polymorphism D

AFLP = Amplified Fragment Length Polymorphism

MCA= Mobile Chlorophyll Analyzer

ISTA= International Seed Testing Association

GE= total germination percentage

GE2=Germination in 2 day

GE4=Germination in 4 day

CoT2= Cotyledon emerged seed count in 6 day

Gst= Standard germination test