

Screening of local Brassica varieties for salt tolerance at germination and seedling establishment stage

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Abstract

An abiotic factor that causes reduction in the productivity of the crops in different parts of the world is salinity which creates changes in different physiological and metabolic processes depending on the duration and severity of the stress condition in which the plant is present. The purpose of the study was to investigate the effect of salt stress on germination and seedling growth of different varieties of *Brassica* (NUYTM, Hyola (2), Abasin-95, NIFA Raya, NIFA Gold, Datura-e-NIFA, NUYTC-13, AARI Canola, NUYTC-16, and NUYTM-17). In this experiment, 10 sterilized seeds of six different varieties of brassica were arranged in petri dishes with 5ml of different concentrations of NaCl (0, 50mM, and 150mM) and each concentration has three replicates. After 7 days the experiment was terminated and different parameters such as (root length, shoot length and vice versa). The current result showed that length of root and shoot, fresh and dry weight of root and shoot, relative water content, vigor index decreased while root shoot ratio, shoot weight ratio, and root weight ratio was increased in NIFA Raya variety. It was concluded that NIFA Raya and NUYTM-17 showed more salt-tolerant than the other cultivars. © 2021 Department of Agricultural Sciences, AIOU

Keywords: Brassica, Metabolic process, Physiological parameters, Salinity stress, Root shoot ratio.

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Introduction

Different environmental conditions e.g., freezing, infrared light, desertification, toxic metals, salts, gaze, low temperature cause a detrimental effect on plant growth and known as abiotic stress factors, and these factors in future pressures increase as a result of global climatic change (Hirayama & Shinozaki, 2010). Stress is defined as any environmental factor that reduces the rate of physiological metabolism, so plants are unable to transform energy into biomass (Pariha et al., 2015). This effect was created as a result of different physiological changes caused due to different temporary modifications in the environment (Schulte, 2014). The salinity stress negatively affects plant growth and development, as a result of a high amount of different minerals (e.g. chlorides and sodium, etc.) absorption through plant roots and this factor also disturbs the nature of soil as well, by increasing the addition of salts which lead to acidify the soil nature (Pariha et al., 2015).

The mustard family is known as *Brassicaceae* in the economically-important family with 372 genera and 4060 accepted-species. This family contains annual, biennial to perennial herbaceous-plants, shrubs, and woody plants distributed across the world mainly in alpine and temperate regions. Most flowers of this family contain corolla in cross-

shaped (four petals are arranged in a cross shape) (Cartea et al., 2011). *Brassica juncea* is a common member of this family and mostly grown as a seed in different countries of the world, with a high level of glucosinolates and increased erucic acid content, but comparatively low content of oil (Gingera et al., 2003). *Brassica napus* is another common member of this family with yellow flowers and known as oilseed rape. This plant is an oil cash-crop with nutritional-importance, producing biofuel, food, and different industrial compounds e.g., different solvents and adhesives (Allender & King, 2010).

Salinity is a major threat to crops, so the scientists are searching for different varieties and techniques to neglect the effect of salinity on soil and water that uptake by plants or manipulate the resistant varieties against the salinity stresses. These strategies include the use of different trehalose, production of transgenic plants through a breeding program. Trehalose is a group of sugars that could protect different bio-molecules against many environmental stresses. This trehalose is present in different bacteria, fungi, and in any stress-tolerant higher plants. Accumulation of these trehaloses improves salt and drought tolerance in plants (Penna, 2003). Different salt-tolerant genotypes/lines are produced through a conventional breeding program to cope with this problem and plants are more efficiently grown under saline/drought conditions (Ashraf et al., 2008). Efficient growth of these genotypes as compared to conventional varieties depends upon an understanding of genetics, physiology, and molecular biology of salt tolerance of plants (Foolad, 2004). Different salt/drought tolerant plants have been developed to cope up and to permeate these stresses. Different salt-tolerant transgenic tobacco plants have been developed by transferring those genes encoded with glycine-beta. In the same way, transgenic salt resistant and low-temperature resistant Arabidopsis lines/genotypes are produced by transferring the genes in chloroplast code involved in the production of glycine-betaine (Sakamoto & Murata, 2000). Currently, different local brassica varieties have been evaluated for salt tolerance at the germination and seedling growth stage.

Materials and Methods

Seeds of Ten different varieties of Brassica (NUYTM, Hyola (2), Abasin-95, NIFA Raya, NIFA Gold, Datura-e-NIFA, NUYTC-13, AARI Canola, NUYTC-16, and NUYTM-17) were obtained from Agriculture Research Institute, TARNAB Peshawar. Germination and seedling growth experiment was performed in the Plant Physiology Laboratory, Department of Botany, Abdul Wali Khan University Mardan, Pakistan. Seeds of six wheat cultivars were sterilized with 0.1% mercuric chloride solution for 1 minute and washed thoroughly three times with distilled water. Sterilized plates lined with two layers of filter paper. Five seeds of ten Brassica cultivars were placed in each sterilized Petri plate. Then 5 ml of NaCl concentrations (50mM, 150mM) was applied in each Petri plate whereas 5ml distilled water was applied for the control treatment. Each treatment was replicated three times. All replicates were kept in an incubator at 25°C for germination. After 24 hours germinated seeds were counted. After 8 days, the experiment was terminated and germination percentage, seedling growth, seedling length, seedlings biomass relative water content, vigor index, RSR, SWR, and RWR were recorded. The average value of shoot length and root length were recorded in cm, after measuring the length of root and shoot, the seedlings were separated and seedlings fresh weight was measured and then plant samples were kept in

the oven at 50° C for 2 days and then dry weight was recorded.

Relative water content

Relative water content (RWC) was determined and calculated through a method described by Barrs and Weatherly (1962).

RWC (%) =
$$\frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Vigor index

Seedling vigor index (VI) was calculated in experimental seedling through a method described by Abdul-Baki and Anderson (1973):

Vigor Index (VI) = (Mean root length + Mean shoot length) × germination percentage

Different ratios

Different ratios in experimental seedlings were calculated through different formulas, described by Hunt (1982):

Root shoot ratio (RSR) =	Root dry weight
	Shoot dry Weight
Shoot weight ratio (SW	$(R) = \frac{Shoot dry weight}{1}$
	, Total dry weight
Root weight ratio (RWR)	Root dry weight
	= Total dry weight

Stress tolerance index

Stress Tolerance Index (STI) of different parameters of experimental seedling were calculated through different formulas described by Ashraf and Harris (2004):

Plant height stress tolerance index (PHSI) = $\frac{\text{Plant hight of stressed plant}}{\text{Plant hight of control plant}} \times 100$
Root length stress tolerance index (RLSI) = $\frac{\text{Stress plant radical extension}}{\text{Control plant radicle extension}} \times 100$
Shoot fresh weight stress tolerance index (SFSI) = $\frac{\text{Stressed plants sprout fresh mass}}{\text{Control plant sprout fresh mass}} \times 100$
Root fresh weight stress tolerance index (RFSI) = $\frac{\text{Stressed plant root fresh mass}}{\text{Control plant root fresh mass}} \times 100$
Shoot dry weight stress tolerance index (SDSI) = $\frac{Stressed \ plant \ shoot \ dry \ mass}{Control \ plant \ shoot \ dry \ mass} \times 100$
Root dry weight stress tolerance index (RDSI) = $\frac{Stressed \ plants \ sprouts \ dry \ mass}{Control \ plant \ root \ dry \ mass} \times 100$

Reduction percentages

Reduction percentages of different parameters of experimental seedlings were calculated through different formulas as described by Raun et al., (2002):

Shoot length reduction percentage (SLRP) =
$$\frac{1 - (\text{Shoot length at salt})}{\text{stress}} \times 100$$
Root length reduction percentage (RLRP) =
$$\frac{1 - (\text{Root length at salt stress})}{\text{Root length control}} \times 100$$
Fresh weight reduction percentage (FWRP) =
$$\frac{1 - (\text{Fresh weight at salt stress})}{\text{Fresh weight controll}} \times 100$$
Dry weight reduction percentage (DWRP) =
$$\frac{1 - (\text{Dry weight at salt stress})}{\text{Dry weight control}} \times 100$$

Statistical analysis

Data analysis was carried out through SPSS (Version 21) statistical software, where one-way analysis of variance (ANOVA) was applied while mean values were compared through Duncan's Multiple Range Test (DMRT) at a 5% probability level.

Result and Discussion

Germination %

Seed germination and seedling establishment considered as most critical stages in plant life cycle, because it determines the fact that whether these seedlings are able to survive in their local environment or not (Bajji et al., 2002). Generally, high salt concentration cause reduction in seed germination process due to presence of low osmotic potential around the seed preventing water uptake by seed (Welbaum et al., 1990). In the present investigation, the effect of different salt concentrations (50 mM and 150 mM NaCl) on brassica cultivars showed significant increase in NUYTM, Abasin-95, and NIFA Gold while NIFA Raya and Datura-e-NIFA showed a reduction in germination percentage as compared to control. Varieties Hyola-2, NUYTC-13, AARI Canola, NUYTC-16, and NUYTM-17 showed the equal percentage of germination in control and different saline concentrations (Fig. 1). Experiments of Hamid et al. (2008) confirmed current findings of retarded germination percentage under different salinity levels, while others explained the fact that retarded germination process with low root emergence was due to osmotic effect caused by stress condition and it is harmful to plants to make them unable to maintain the proper nutritional balance required for the normal and healthy growth of plant while others also observed low germination percentage due to high osmotic potential as high NaCl level applied in the growth medium. This increased osmotic potential causes deteriorated seed and slower germination with a high level of sodium and chloride ions accumulation, with the help of low absorption of water, specific ions toxicity with imbalanced ions/nutrients uptake through roots (Cokkizgin, 2012).



Fig. 1 Effect of salt stress on germination of different Brassica varieties

Shoot length

The current results regarding shoot length, at 50 mM NaCl concentration it showed 23%, 21%, 5%, 27%, 34%, 23%, 25%, 27%, 14% and 30% reduction while at 150 mM NaCl level same parameter exhibited 42%, 29%, 46%, 36%, 50% 32% 31%, 42%, 47% and 56% reduction in NUYTM, Abasin-95, Hyola-2, NIFA Raya, Datura-e-NIFA, NIFA Gold, NUYTC-13, AARI Canola, NUYTC-16 and NUYTM-17 respectively as compare to control seedlings.

Hyola-2 showed more tolerance in shoot length as compared to other varieties. A continuous decrease was noted in all varieties as salinity level increases (Fig. 2). Kapoor and Pande (2015) also observed the same findings of reduced shoot length of fenugreek when raised under different salinity regimes while others also observed reduced shoot length in different plant species, when exposed to saline medium, with the reason that translocation and efficacy of adjustment of photosynthetic products were inhibited.



Fig. 2 Effect of salt stress on plant height of different Brassica varieties

Root length

Tolerance of any plant towards salt stress depends on the efficiency of its root system to absorb different ions especially potassium and sodium, then discrimination and their translocation to the whole plant body (Khan et al., 2000), while some observed that sodium ion mainly retains in root portion as compared to shoot and (Khan et al., 2000) also confirmed the same statement in their studies. According to results regarding root length, at 50 mM NaCl concentration it showed 35%, 9%, 15%, 15%, 28%, 16%, 33%, 36%, 5% and 22% reduction while at 150 mM NaCl level same parameter exhibited 63%, 28%, 53%, 36%, 39%, 20%, 56%, 21%, 14% and 27% reduction in NUYTM,

Abasin-95, Hyola-2, NIFA Raya, Datura-e-NIFA, NIFA Gold, NUYTC-13, AARI Canola, NUYTC-16 and NUYTM-17 respectively as compare to control seedlings (Fig. 3). NYC-16 showed more tolerance in root length as compared to other varieties. Hossain et al. 2006) worked on different crops and observed the same results so they confirmed that the toxicity of salt negatively affects plant growth. As a result of high concentration of NaCl in the growth medium, it slows down/stop the root elongation process or in some cases, root production stopped reported in other studies while others also worked on maize plant and raised them under salt stress condition and observed reduced length of roots as compare to non-saline condition.



Fig. 3 Effect of salt stress on root length of different Brassica varieties

Fresh biomass

Under environmental stresses especially salt stress reduce the growth and yield of different crops. This reduction in plant growth might be a result of low absorption and translocation rate of different ions e.g. nitrates in plants which in-turn lower the synthesis of nitrogen compounds, on the other hand, sodium ions level increased in high salt level (Hamid et al., 2008). According to results regarding fresh weight at 50 mM NaCl concentration it showed 21%, 30%, 33%, 40%, 26%, 24%, 54%, 8%, 37%, and 21% reduction while at 150 mM NaCl level same parameter exhibited 35%, 35%, 49%, 48%, 31%, 49%, 61%, 78%, 55% and 43% reduction in NUYTM, Abasin-95, Hyola-2, NIFA Raya, Datura-e-NIFA, NIFA Gold, NUYTC-13, AARI Canola, NUYTC-16 and NUYTM-17 respectively as compared to control seedlings. AARI Canola and Datura-eNIFA showed more tolerance in fresh weight as compared to other varieties (Fig. 4,5,6). Houle, 2001) observed in his experiments that germination of the seed, emergence of seedling, and growth decreased as the salt level increased in the growth medium. Another study observed 48 and 59% reduction in bean plants while 14% reduction in cotton plants in biomass production when these plants grew in different salt stress levels. According to Chien et al., 2009), under salt stress conditions accumulation of different toxic ions e.g. sodium, sulfates, and chlorides cause inhibition of nitrate uptake in plant roots. Others worked on calendula plant and observed reduction in biomass production of root and shoot under salt stress condition while other studies discussed the reason of reduction in the growth of plant under stress condition due to creation of osmotic and ionic stress with a harmful effect on plant growth.



Fig. 4 Effect of salt stress on shoot fresh weight of different Brassica varieties



Fig. 5 Effect of salt stress on root fresh weight of different Brassica varieties



Fig. 6 Effect of salt stress on total fresh biomass of different Brassica varieties

Dry biomass

It is well documented when plants grown under salt stress reduction in fresh and dry biomass of seedlings was observed which is due to the reduction of water absorption through seedlings (Ashraf & Bhatti, 2000). According to results regarding dry weight, at 50 mM NaCl concentration it showed 75%, 44%, 87%, 25%, 62%, 54%, 91%, 34%, 25% and 55% reduction while at 150 mM NaCl level same parameter exhibited 91%, 83%, 90%, 35%, 72%, 89%, 94%,

83%, 85% and 65% reduction in NUYTM, Abasin-95, Hyola-2, NIFA Raya, Datura-e-NIFA, NIFA Gold, NUYTC-13, AARI Canola, NUYTC-16 and NUYTM-17 respectively as compare to control seedlings (Fig. 7,8,9). NUYTC-16 and NIFA Raya showed more tolerance in dry weight as compared to other varieties. Hamid et al. (2008) worked on hybrids of maize (*Zea mays* L.), sugarcane, and wheat respectively under salt stress conditions and observed reduction in root and shoot length with fresh and dry biomass of seedlings under stress conditions.



Fig.7 Effect of salt stress on shoot dry biomass of different Brassica varieties



Fig. 8 Effect of salt stress on root dry biomass of different Brassica varieties



Fig. 9 Effect of salt stress on total dry biomass of different Brassica varieties

Relative water contents

Relative water contents (RWC) is considered an important criterion to measure the effect of different environmental stresses especially salinity and drought. This is a reliable parameter to measure the water status of the plant, while leaf relative water content is considered as a balance between the water status of the plant and the rate of transpiration with direct relation to cell volume of the same plant. McCaig and Romagosa (1991) considered this parameter as an index that is useful for the determination of leaf and plant water status under any normal and stressed condition. According to results regarding relative water content, at 50 mM NaCl concentration it showed 12%, 11%, 11%, 6%, 10%, 20%, 14%, 14%, 20% and 8% reduction while at 150 mM NaCl level same parameter exhibited 28%, 32%, 23%, 35%, 29%,

27%, 37%, 36%, 30% and 23% reduction in NUYTM, Abasin-95, Hyola-2, NIFA Raya, Datura-e-NIFA, NIFA Gold, NUYTC-13, AARI Canola, NUYTC-16 and NUYTM-17 respectively as compare to control seedlings (Fig. 10). NIFA RAYA, Hyola-2, and NUYTM-17 showed more tolerance in relative water content as compared to other varieties. (Awasthi et al., 2016) worked on plants under salt stress and observed a great negative effect on relative water content (RWC) of leaf and plant, while other studies observed the same results when plants were kept under drought conditions with PEG and NaCl stress. Reduction in this parameter under any stressed condition was observed due to low water absorption through the root system due to root injury coupled with low substrate water potential (Garg & Singla, 2009).



Fig. 10 Effect of salt stress on relative water contents of different Brassica varieties

Root shoot ratio

Different morphological parameters e.g., shoot length, root length, leaf number, and proportion of root/shoots as well as plant biomass were negatively affected by induction of any environmental stress condition (Gama et al., 2007). According to results regarding root shoot ratio, at 50 mM NaCl concentration it showed 227%, 16%, 37%, 251%, 69%, 107%, 58%, 66%, 94% and 34% promotion while at 150 mM NaCl level same parameter exhibited 371%, 131%, 100%, 733%, 227%, 257%, 85%, 141%, 281% and 131% promotion in NUYTM, Abasin-95, Hyola-2, NIFA Raya, Datura-e-NIFA, NIFA Gold, NUYTC-13, AARI Canola, NUYTC-16 and NUYTM-17 respectively as compare to control seedlings. NIFA Raya showed more tolerance in root shoot ratio as compared to other varieties (Fig. 11). When different plant species were grown under salt stress, the plant exhibited higher root dry biomass than shoot dry biomass, resulting in an enhanced root to shoot ratio which is considered as an improvement in source to sink ratio for different nutrients and water. In a study of Moud and Maghsoudi (2008) on different crops under salt stress conditions, they observed high values of root to shoot ratio with inhibitory effects of applied stress. This reflects a higher ratio of absorption of water through roots and area of transpiration to the characteristics of the plant and uses for the plant under a dry-land environment when the plant grows for a long time during other stages of growth.



Fig. 11 Effect of salt stress on root shoot ratio of different Brassica varieties

Shoot weight ratio

When the plant grows, the relative length of shoot and root of seedling closely related to its total biomass production, while under stress condition this thing is considered as plant tolerance against stress in which it is present and it is directly related to the high absorption rate of potassium ion with a low concentration of sodium in shoots which positively correlated with K/Na ratio and Ca/Na ratio. Yurtseven et al. (2005) also observed the same results of low biomass production in studied plants when raised under increased salinity level. According to results regarding shoot weight ratio, at 50 mM NaCl concentration it showed 8%, 12%, 14%, 18%, 30%, 27%, 30%, 22%, 25% and 45% reduction while at 150 mM NaCl level same parameter exhibited 25%, 21%, 23%, 49%, 53%, 43%, 53%, 49%, 56% and 53% reduction in NUYTM, Abasin-95, Hyola-2, NIFA Raya, Datura-e-NIFA, NIFA Gold, NUYTC-13, AARI Canola, NUYTC-16 and NUYTM-17 respectively as compare to control seedlings (Fig. 12). NUYTM and Abasin-95 showed more tolerance in shoot weight ratio as compared to other varieties. Munns (2002) observed a higher reduction rate of shoot-lengths as compared to root length as a common feature in different studied crops. Jamil et al. (2005) worked on three *Brassica* species including *Brassica oleracea* and *Brassica napus* under salt stress up to 14.10 dS m⁻¹ and observed the same results and this reduction was caused due to toxicity of ions with inhibitory effect. Further, the reduction in root and shoot length occur in plants due to low water uptake by seed. Different researchers observed the same results of reduction in seedling lengths with biomass as a common phenomenon in different crops grown under salt stress conditions.



Fig. 12 Effect of salt stress on shoot weight ratio of different Brassica varieties

Root weight ratio

Generally, under saline environments, in plants root plays an important role in the growth of shoots which is due to translocation of salts and different ions actively from root to shoots (Asaadi, 2009). Length of roots is considered an important parameter in response to drought stress in different plant cultivars, so cultivar which had longer roots had greater resistance ability for drought. According to results regarding root weight ratio, at 50 mM NaCl concentration it showed 35%, 29%, 24%, 27%, 34%, 48%, 76%, 53%, 47% and 23% reduction while at 150 mM NaCl level same parameter exhibited 67%, 60%, 46%, 47%, 55%, 58%, 84%, 68%, 81% and 63% reduction in NUYTM, Abasin-95, Hyola-2, NIFA Raya, Datura-e-NIFA, NIFA Gold, NUYTC-13, AARI Canola, NUYTC-16 and NUYTM-17. (Fig. 13). respectively as compare to control seedlings. NUYTM-17 and Hyola-2 showed more tolerance in root weight ratio as compared to other varieties. Kapoor and Pande (2015) observed reduction in shoot biomass as well as root biomass as a result of restricted water absorption through roots under different environmental stress conditions. Further, this restricted absorption process resulted in osmotic stress-induced through sodium chloride (Sikha et al., 2014) while another study on a medicinal plant investigated, ajwain (*Trachyspermum ammi* L.) and grew it under stress condition and observed reduced root and shoot ration of the studied plant.



Fig. 13 Effect of salt stress on root weight ratio of different Brassica varieties

Vigor index

Under stress factors, especially drought, and salt stress, the vigor index (VI) of the seedling is considered and used as a tolerance index to measure the effect of applied stress on the growth of seedlings (da Silva Oliveira & Steiner, 2017). Kapoor and Pande (2015) also considered this parameter to evaluate the effect of stress environment and confirmed harmful effects of stress on plants using this parameter. Bewley et al. (2013) defined this parameter as the measure of damaging ability accumulation that causes viability decline of any seed or seed that is unable to germinate/die. (Fig. 14). According to results regarding vigor index, at 50 mM NaCl concentration it showed 32%, 24%, 15%, 30%,

33%, 17%, 30%, 30%, 34% and 14% reduction, while at 150 mM NaCl level same parameter exhibited 44%, 33%, 48%, 50%, 48%, 46%, 47%, 61%, 46% and 38% reduction in NUYTM, Abasin-95, Hyola-2, NIFA Raya, Datura-e-NIFA, NIFA Gold, NUYTC-13, AARI Canola, NUYTC-16 and NUYTM-17 respectively as compare to control seedlings. NYUTM-17 showed more tolerance in vigor index as compared to other varieties. Cokkizgin (2012) worked on different plant species and grew them in salt stress conditions, and observed low seed vigor index as salinity level increased in the growth medium. Further, others explained that a lower level of vigor index of any seed is a result of reduced water potential and negative impact of specific ions.



Fig. 14 Effect of salt stress on vigor index of different Brassica varieties

Salt tolerance index

It is a well-known and common phenomenon that salt application on plants causes a reduction in plumule and radicle length, biomass, and plant height. When different varieties of Brassica were grown under salt stress, genotypic-variation was evaluated after the estimation of different physiological parameters with the help of different growth parameters. All physiological indices (PHSI, RLSI, SFSI, RFSI, SDSI, and RDSI) were decreased due to salinity showing that the growth was significantly affected by salt stress. At 50 mM NaCl concentration Hyola-2 showed the highest value of PHSI, NUYTC-16 Bathor showed a high value of RLSI, SFSI, SDSI, DBSI, AARI Canola showed high values in RFSI, FBSI, and RDSI. At 150 mM NaCl concentration, Abasin-95 showed a high value of PHSI, NUYTC-16 showed a high value of RDSI, NUYTM-17(Fig. 15). showed high values of SFSI and FBSI, NIFA Raya showed high values of SDSI, DBSI, Datura-e-NIFA showed high value of RFSI and NIFA Gold showed the highest value of RDSI. The varieties IAARI Canola, NUYTC-16, NIFA Raya, NUYTM-17 had the higher tolerance indices reflecting greater salt tolerance. Different studies by different researchers confirmed this hypothesis that increasing salt stress levels in the growth medium resulted in reduced and stunted growth of plants (Yurtseven et al., 2005). Ashraf et al. (2008) worked on sorghum and other crop varieties and observed a reduction in growth after application of salt in the growth medium, they explained as this reduction was a result of the accumulation of sodium ions in leaves while among different varieties, tolerant varieties had low sodium absorbing ability that sensitive cultivars.



Fig. 15 Salt tolerance index of different Brassica varieties

Conclusion

Based on the present research, NYUTM-17 was identified as the most salt-tolerant variety followed by NIFA Raya. It is also outlined from current research that different parameters (germination percentage, length, and biomass of root and shoot, vigor index, RWC) were considered as major components to identify salt tolerance character in varieties during germination and seedling establishment stages.

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