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Physiological performance of soybean germination and seedling growth under salinity stress



Soyada tuzluluk stresinin çimlenme ve fide gelişimi üzerine fizyolojik tepkilerinin saptanması

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ABSTRACT

One of the most widespread agricultural problems in arid and semiarid regions is soil salinity. Soybean is one of the most important fabaceae plants. The objectives of this investigation were concentrated on the influence of three saline concentrations (Control, 25 and 50 mM NaCl) on some physiological parameters during germination and seedling stage of five soybean cultivars. The results were revealed a large variability within cultivars for salt tolerance at the early growth stages. Germination percentages, chlorophyll content, leaf number and plant height were significantly reduced with increasing salinity concentrations. The Japanese cultivar (TSU) and Egyptian cultivar (Giza-111) was less affected by salinity stress than other cultivars. Also, TSU and Giza -111 maintained a higher germination percent, dry weight and relative water content than other cultivars with increasing of salinity concentrations. Salinity stress induced a significant increase in seedling tissue sodium (Na+) in all cultivars. However, TSU and Giza-111 maintained lower Na+ both higher potassium (K+) and proline accumulated at the higher saline conditions than other cultivars. In this study, exhibited higher levis of tolerance to salinity compared to the other cultivars during germination and seedling stage.

Keywords: Salinity, Germination, Proline, Sodium, Potassium

ÖZET

Tuzlaşma, kurak ve yarıkurak tarım alanların en yaygın sorunlarından birini oluşturmaktadır. Soya, önemli bir baklagil bitkisidir. Bu çalışmada, üç farklı tuz konsantrasyonunun (Kontrol, 25 and 50 mM NaCl), beş soya çeşidinin çimlenme ve fide özelliklerine fizyolojik etkileri araştırılmıştır. Çeşitler, erken gelişme döneminde tuz toleransı yönünden büyük farklılıklar göstermiştir. Çimlenme oranı, klorofil miktarı, yaprak sayısı ve bitki boyu artan tuz konsantrasyonu ile birlikte önemli düşüşler göstermiştir. Japon TSU ve Mısır Giza-111 çeşitleri tuz stresinden diğer çeşitlere göre daha az etkilenmişlerdir. Ayrıca, TSU ve Giza-111 çeşitleri, artan tuz consantrasyonlarında diğer çeşitlere oranla daha yüksek, çimlenme oranı, kurumadde ve oransal su içeriği göstermiştir. Tuz stresi, çeşitlerin tamamında fide dokusunda Na+ artışını teşvik etmiştir. Buna karşın TSU ve Giza-111 çeşitleri, tuzlu koşullarda diğer çeşitlere oranla daha düşük Na+ ve daha yüksek K+ ve prolin biriktirmişlerdir. Bu çalışmada, TSU ve Giza-111 çeşitlerinin çimlenme ve ilk fide döneminde diğer çeşitlere oranla tuza daha toleranslı olduğu saptanmıştır.

Anahtar sözcükler: Tuzluluk, Çimlenme, Prolin, Sodium, Potasyum

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1. Introduction

Salinity is a major environmental constraint to crop productivity throughout the arid and semi-arid regions of the world [1]. Salinity has reached a level of 19.5% of all irrigated-land (230 million ha of irrigated land, 45 million ha are salt-affected soils) and 2.1% of dry-land (1500 million ha) of dry land agriculture, 32 million are salt-affected soils) agriculture worldwide. Seed germination is a major factor limiting the establishment of plants under saline conditions. Salinity may cause significant reductions in the rate and percentage of germination, which in turn may lead to uneven stand establishment and reduced crop yields [2]. Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment. Germination failures on saline soils are often the results of high salt concentrations in the seed planting zone because of upward movement of soil solution and subsequent evaporation at the soil surface [3]. Seed germination, seedling emergence, and early survival are particularly sensitive to substrate salinity .Successful seedling establishment depends on the frequency and the amount of precipitation as well as on the ability of the seed species to germinate and grow while soil moisture and osmotic potentials decrease [4]. However, soybean (Glycine max (L.) Merrill), an important crop that provides fatty acids and proteins for humans and animals, is a salt-sensitive crop [5].

Soybean is a strategic crop plant grown to obtain edible oil and forage. High sensitivity to soil and water salinity is one of the biggest problems with soybean crop. Results have indicated that salinity affects growth and development of plants through osmotic and ionic stresses. Because of accumulated salts in soil under salt stress condition plant wilts apparently while soil salts such as Na+ and Cl- disrupt normal growth and development of plant [6, 7, 8]. Soybean was selected as the model species for this work because of its known sensitivity to saline conditions and its importance in world agriculture. The present study was conducted to evaluate the effect of salinity on seed germination and seedling growth of five soybean cultivars under salinity conditions to sort out and determine the variety of soybean tolerant to salinity stress and find out its importance under salinity stress.

2. Materials and Methods

2.1. Plant Material and Culture Conditions

The experiment was conducted at the plant nutritional physiology laboratory, Hiroshima University, Japan.

The widely adapted soybean (Glycine max L.) cultivars from Egypt (Giza-111, Giza-82, Giza-35) and Japan (Tsurvnoko (TSU) and Fukuyukaka (FU). The seeds of all cultivars were surface sterilized with 5% Thiophanatemethyl for 5 mins and air dried. The seeds were sown in to plastic filled with (1 kg) of mixture of granite regosol soil and perlite (2:1:1, v/v). Pots were kept under greenhouse conditions. Plants were irrigated with nutrient solution (200 ml pot⁻¹) twice a day (at 10:00 a.m. and 15:00 p.m.) with contained 0 (Control), 25 and 50 mM NaCl until water drained from the bottom of the pot. The basal nutrient solution contained 8.3 mM NO₃-N, 0.8 mM NH₄-N, 0.5 mM P₂O₅, 2.2 mM K₂O, 0.7 mM MgO, 2.1 mM CaO, 11 µM MnO, 5 µM B₂O₂ and 13 µM Fe. The experiment was designed as A Completely Randomized Plot Design with five replicates and located randomly in the greenhouse and experimental was ended 21 days from the sowing. After 3 weeks from planting date the following growth porters were recorded; plant height, chlorophyll content, number of leaves, fresh and dry weight (shoot, root), relative water content, germination percentage, salt tolerance, ions accumulation (Na, K) and Proline content.

2.2. Measurement of Growth

Just after application of treatments and at 21 d after treatments imposition, plants in each pot were sampled and separated into the shoot and roots. Shoots were wiped with tissue towel paper to avoid moisture and their fresh weights were measured. Roots were washed gently with tap water for a few minutes and wiped with tissue towel paper and also measured fresh weight. The fresh samples were kept frozen in liquid nitrogen, then dried at -82° C in a freeze drier for 72 h and measured the dry weight. Dry samples were ground into fine powder using a vibrating sample mill (Model T1-100, Heiko Co., Ltd., Japan) for chemical analysis. The leaves were oven dried at 80°C for 72h and taken dry weight.

2.3. Measurement of Relative Water Content

The fresh weight of some part of leaf was measured and kept immersed in distilled water for 24h at room temperature in the light. The turgid weight of leaf was then measured and afterwards oven-dried at 80°C for 72h in order to take dry weight. The fresh, turgid and dry weights of the leaf segments were used to determine the following parameters:

 $Relative Water Content (RWC) = \frac{Fresh weight - Dryweight}{Turgid weight - Dryweight} x100$ (1)

2.4. SPAD Chlorophyll Meter Reading

Leaf chlorophyll concentration was measured with hand held chlorophyll meter (SPAD-502, Minolta, Japan). SPAD values were recorded from the fully expanded all leaves counted in the seedling.

2.5. Germination Percent

The emergence of root/shoot from seeds was taken as an index of germination (%) which was recorded daily for up to 15 days. After that salt tolerance was calculated by [9].

2.6. Salt tolerance

Salt tolerance (%) =
$$\frac{A}{B} \times 100$$
 (2)

Where A; germination in treated seedlings, B; germination in control plants.

The seedling growth evaluation was carried out by [10].

2.7. Measurement of Na⁺ and K⁺ Concentrations

Seedling tissue Na⁺ and K⁺ concentrations were determined after digestion by nitric acid-hydrogen peroxide, using a flame photometer (ANA 135, Eiko Instruments Inc., Tokyo).

2.8. Measurement of Proline

Dried ground samples used for proline determination were transferred to vials subjected to methanol extraction and stored in the dark at 4°C. Proline was determined spectrophotometrically following the ninhydrin method described by [11] using L-proline as a standard and then determined using spectrophotometer (U-3310, Hitachi, Ltd. Tokyo, Japan)

2.9. Statistical Analysis

Data were examined by one-way ANOVA analysis of variance. Multiple comparisons of means of data among different salinity treatments within the plants were performed using Duncan's test at the 0.05 significance level (all tests were performed with SPSS Version 16.0 for Windows).

3. Results and Discussion

Soybean is the important seed legumes in the tropics and subtropics. With the scarcity of good land, due to increasing demand for cereal based food, the legume cultivation is pushing to the marginal soils including saline soils. A little research attention has been paid for the improvement of legumes as well as for the development of their production practices in the saline soils.

3.1. Plant height

Salinity induced reduction in plant height is well known [12]. Applied salinity of 0, 25, and 50 mM NaCI on soybean observed that plant height of this crop was decreased with the increasing levels of salinity (Table1). The percent reduction of plant height was greater in

Egyptian cultivars than Japanese cultivars in saline stress which decreased almost double in all genotypes. Among the genotypes, TSU cultivars registered the highest values by showing less reduction in plant height in saline conditions. In a study in Vietnam, [13] also noticed a reduction in plant height of soybean varieties due to salinity, [14] also noticed a decrease in plant height in *Vigna radiata, V. mango and Cyaniopsis tetragonoloba* with increasing salinity levels from 3-18 mS/d. [15] conducted pot experiment at BSMRAU with 4 levels of salinity (0, 50, 75 and 100 mm NaCl) considering blackgram and mungbean as test crops. He observed that plant height of both the crops significantly decreased with increasing salinity, and mungbean was more sensitive to salinity than black gram.

3.2. Leaf Chlorophylls (SPAD)

In this study Salinity induced reduction in Leaf Chlorophyll content and the percent reduction of chlorophyll content was greater in Egyptian cultivars

Table 1: The effects of salinity on plant height, number of leaves, Chlorophyll content and R.W.C. of soybean cultivars.

Characters	Cultivars					Salinity levels "Nacl mM"			Interaction
	TSU	FU	Giza-111	Giza-82	Giza-35	Control	25mM	50mM	
Plant height (cm)	16.a	11.92c	15.85b	12.14c	11.46d	18.4a	14.2b	8.4c	**
Chlorophyll (SPAD)	39.7a	34.1c	38.2b	37.9b	34.5b	41.4a	37.2b	34.4c	**
Number of leaves	5.2a	4.6b	4.75b	4.4c	4.2c	5.2a	4.7b	4.1c	*
Relative Water content (%)	93a	87c	90b	85c	83d	89a	74b	55c	**

*, ** indicate P < 0.05, P < 0.01 and not significant, respectively. Means within the same column of each factor followed by a common letter are not significantly different at the 5% level, according to Duncan's multiple range tests

than Japanese cultivars in saline stress which decreased almost double in all genotypes. Among the genotypes, TSU cultivar registered the highest values by showing less reduction in chlorophyll in saline conditions (Table 1). The physiological basis of salt tolerance of Soybean was assessed in a salinized sand culture. It was found that increasing NaCl concentration in the rooting medium significantly decreased the chlorophyll content [16]. Decreasing chlorophyll content index (CCI) of soybean leaves with increasing salinity could be related to increasing the activity of chlorophyll degrading enzyme: chlorophyllase [17], and the destruction of the chloroplast structure and theinstability of pigment protein complexes (Table 1) [18].

3.3. Number of Leaves

The influence of salt stress on the number of leaves per plant was significant (Table 1). The highest number of leaves (5.2) was found at control treatment and the lowest (4.1) one was found at highest salinity levels. This result showed that number of leaves plant ⁻¹ decreased gradually with increasing salinity in comparison to that control. Among cultivars, TSU cultivar produced the highest number of leaves /plant in comparison with the other cultivars under salinity conditions. This reduction may be related to enhancing the activity of chlorophyll degrading enzyme chlorophylls [19]. It could be concluded that, ion accumulation in leaves specially chloride and sodium, affect chlorophyll biosynthesis due to its effect on the activity of Fe-containing enzymes, cytochrome oxidize.

3.4. Relative Water Content

The relative water content (RWC) in all cultivars was decreased with increasing NaCl concentration and interaction of salt composition with cultivars. It was found that the reduction of RWC was less in Tsu cultivar and GİZA-111 than other cultivars (Table 1). The decrease in leaf water content that affect the synthesis of hydrolytic enzymes limiting the hydrolysis of food reserves from storage tissues as well as to impaired translocation of food reserves from storage tissue to developing embryo axis [20].

3.5. Shoot Fresh Weight

Salinity has a direct influence on plant biomass production of a crop. The Plant fresh weight of both Japanese and Egyptians groups declined with saline stress (Table 2). Changes of plant dry weight were similar to that of plant fresh weight [21] stated that both shoot and root growths were significantly reduced with the increasing salinity levels in pea. In general, results reported to date have been analyzed from the physiological and metabolic aspects. Salinization is manifested basically as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cell. The presence of sodium ions may cause membrane depolarization, which may lead to the disruption not only of ion selectivity but also of mechanisms of ion uptake [22].

3.6. Root Fresh Weight

Salinity has a direct influence on plant biomass production of a crop. The Plant fresh weight of both Japanese and Egyptians groups declined with saline stress. However, TSU and Giza-111 produced significantly greater dry matter than each other's (Table2). Changes of plant dry weight were similar to that of plant fresh weight. [23] reported that root elongation as well as root biomass was significantly reduced in some mungbean genotypes due to salinity.

3.7. Shoot dry Matter

Data regarding Shoot dry weight of cultivars were positively affected by increasing salt treatments. NaCl stress has highly significant soybean. The study revealed that the Shoot dry matter was decreased with the increasing salinity levels. TSU and Giza 111 cultivars produced significantly greater dry matter than each other's cultivars (Table 2). [24], assessed the salt tolerance of two cultivars of mung bean .They found that increasing salt concentration significantly reduced the dry matter, weight of stems in both the cultivars. Reduction in stem dry weight under saline condition was confirmed in mungbean by [25, 26]. Salinity decreased the stem dry mass irrespective of species (blackgram and mungbean) or varieties and the longer exposure to higher salinity was the damage [15]. According to the previous values, the cultivars were arranged as following: TSU > FU > Giza111 > Giza82 > Giza35 (Table 2). Our results are in agreement with the results of other researchers. For example, [27] reported that a negative relationship was detected between vegetative growth parameters and increasing salinity. In same study, shoot dry weight was 52.01 mg per plant at control while it decreased linearly to 25.26 mg per plant at 4000 ppm. The same results were also obtained by other researchers [28].

3.8. Root Dry Matter

Salinity has a direct influence on plant biomass production of a crop. The Plant dry weight of both Japanese and Egyptians groups declined with saline stress. (Table 2). Changes of plant dry weight were

Characters	Cultivars						linity lev NaCl mM	Interaction	
	TSU	FU	Giza-111	Giza-82	Giza-35	Cont.	25mM	50mM	
Shoot f. w.(g/plant)	3.1a	2.1c	2.67b	1.95c	1.84d	2.9a	2.42b	1.58c	**
Root f. w.(g/plant)	1.83a	1.3c	1.65b	1.28c	1.1d	1.88a	1.44b	0.98c	**
Shoot d. w.(g/plant)	0.369a	0.247c	0.349b	0.244c	0.214d	0.391a	0.281b	0.182c	**
Root d. w. (g/plant)	0.12a	0.106b	0.11b	0.101c	0.101b	0.152	0.102	0.066	**

Table 2: The effects of salinity on plant height, number of leaves, fresh, dry weigh (shoot, root) and R.W.C. of soybean cultivars

*.** indicate P < 0.05, P < 0.01 and not significant, respectively. Means within the same column of each factor followed by a common letter are not significantly different at the 5% level, according to Duncan's multiple range tests.

similar to that of plant fresh weight. Root dry weight of cultivars decreased significantly as the levels of salinity increased from 0 to 50 mM NaCl. Thus, the highest root dry weight was determined at control and the lowest root dry weight at the highest salinity level. [23] reported that root elongation as well as root biomass was significantly reduced in some mungbean genotypes due to salinity. [21] stated that both shoot and root growths were significantly reduced with the increasing salinity levels in pea. [29] evaluated the effect of salinity at the early seedling stage of horsegram. The study revealed that the root yield was decreased with the increasing salinity levels. Roots of mungbean were found to be damaged more than those of black gram [15]. Salinity stress companied by increasing soil ions concentration and charging the ions balance cause to decreasing the germinating and plant growth rate. The results of this investigation showed that salinity decreased the germinating percentage, seedling fresh weight, shoot and root length of soybean cultivars. Overall by increasing the salinity level, potassium content of plant tissues decreased, however sodium content of plant tissue increased. These results has conformity with the Chippa and Rana results [30].

3.9. Germination Percentage

The results of seed germination are shown in (Figure1) It was observed that the germination inhibited with NaCl treated. This tendency was observed in the five cultivars, but it was most strongly expressed in GİZA-111 cultivar and TSU, respectively. Decrease of seed germination under conditions of salt stress is due to occur of some metabolically disorders. It seems that, decrease of germination percentage and germination pace is related to reduction in water absorption into the seeds at imbibitions and seed turgescence stages.

In addition, high absorption of Na and Cl ions during seed germination can be due to cell toxicity that finally inhibits or slows the rate of germination and thus decreases germination percentage [31]. In this study, the responses of cultivars to different salt concentrations were found significantly different. This condition caused significant interactions between salt treatments and cultivars. This means that there are genetically differences among cultivars in respect of tolerance to salt stress. However, increasing salinity decreased the germination percentage



Figure 1: Effects of salinity concentrations on germination percent (%) of soybean cultivars.

in all cultivars; some of the cultivars were more tolerant than the others. As the result of this fact, these results are in agreement with the finding of other investigators [32]. Who reported that the rate and percentage of seed germination of chickpea were significantly reduced by increasing salinity levels and the magnitude of the reduction varied among genotypes. The results obtained here show that NaCl stress has significant effects on soybean seed germination and seedling growth. As reported earlier, saline stress interfere to some degree with many vital plant processes. Reported studies using NaCl have shown inhibitory effects on seed germination, seedling growth and biomass of rice (Oryza sativa), maize (Zea mays), bean (Phaseolus vulgaris), alfalfa (Medicago sativa) and sorghum (Sorghum bicolor) as cited by different authors [33, 34, 35, 36, 37, 38].

Salinity affects germination in two ways; there may be enough salt in the medium decrease the osmotic potential to such a point which retard or prevent the uptake of water necessary for mobilization of nutrient required for germination or the salt constituents or ions may be toxic to the embryo. Our results corresponded to these of [9].

3.10. Salinity Tolerance

The salt tolerance of cultivars at the early seedling stage also showed a large genotipic variation. Giza-111 had the highest salt tolerance index .Therefore; Giza-111demonstrated a better tolerance to salt stress than other cultivars. The effects of different salt concentrations on salt tolerance indices of cultivars were of importance. As the salt concentrations increased the salt tolerance indices of cultivars decreased. Therefore, the lowest value of salt tolerance index was determined at 50 mM NaCl (Figure 2). That salt tolerance was directly related to the amount of water absorbed and the delay in germination to the salt concentration of the medium and the salt tolerance of plants varies with the type of salt and osmotic potential of the medium. Inhibition of germination due to salinity as suggested in previous reports. [39] is attributed to a decrease water content, that affect the synthesis of hydrolytic enzymes limiting the hydrolysis of food reserves from storage tissues as well as to impaired translocation of food reserves from storage tissue to developing embryo axis [20].

3.11. Concentrations of Ions in Plant Parts

3.11.1. Sodium

As shown from Figure 3, sodium accumulation in different plant parts increases with increasing

salinity levels. The Na⁺ content in all cultivars increased with increasing NaCl concentration; this tendency was observed in the five cultivars, but it was decreased most decreased expressed in TSU and Giza-111 cultivar (Figure 3). Salt tolerant variety accumulated less amount of Na^y in most of the plant parts compared to susceptible one, except roots. This is in agreement with the results of in chickpea [40]. Among the plant parts, roots retained maximum Na⁺, which was lower in stem and the lowest in leaf in chickpea genotypes [41, 42], studied soybean, wheat, maize and cotton and suggested that Na+ concentration increased with the increase in salinity level in all of these plants. Root Na+ content of cotton which is a salt stress tolerant plant was more than soybean root indicating preservation of Na+ in cotton root and lack of Na+ transportation to shoot. Data of this study showed



Figure 2: Effects of salinity concentrations on salinity tolerance (%) of soybean cultivars.

negative correlation between Na+ concentrations in shoot of soybean and positive correlation between K+ concentration and these parameters.

There is a relationship between potassium decreasing and sodium increasing in seedling tissue with sensivety to salinity. Sodium affected the cell membrane permeability, deconstructed the cell membrane and destroyed the selectivity property [43,44].

3.11.2. Potassium

In general, K⁺ was decreased by salinity in most of the plant parts in soybean cultivars. The K⁺ content was slightly decreased by NaCl treatment in TSU and Giza111 cultivar. In contrast, it was severely decreased in other cultivars (Figure 4). K⁺ concentration in root tissue was decreased though

Na+ and Cl⁻ concentrations were generally increased with an increasing salinity level, [45, 29] observed in horsegram and greengram that K4concentration in roots decreased and in shoots increased under salt stress conditions. It is reported that under saline conditions plant cells utilize K+ as a metabolite to maintain turgor to escape from osmotic shock [46, 47]. The tolerant genotypes of peas showed increasing while the sensitive ones showed decreasing tread in potassium accumulation under saline conditions [48]. Researchers suggested



Figure 3: Effects of salinity concentrations on Na concentrations of soybean cultivars.



Figure 4: Effects of salinity concentrations on K concentrations of soybean cultivars.

that K+ concentration observed in salt stress tolerant plants were more than that of susceptible cultivars led to decreased Na+toxicity. Increased Na+ content led to decrease in seed germination level and seedling fresh weight in such plants [42,49,50]. [51], working on Triticale cultivars suggested that K+ / Na+ ratio decreased with the increase in salt stress level in growth environment in all of investigated cultivars, however, more increase value was observed among salt stress susceptible plants and reported decreased K+ absorption inpresence of NaCl as the cause of this observation.

3.11.3. Proline

Proline is considered to be the most important organic compatible osmolyte and it is also a protectant of biological macromolecules in the protoplasm. In general, proline accumulation correlates closely with the intensity of the osmotic stress [52] caused by salt, and others stresses. Proline content in all cultivars increased with increasing NaCl concentration (Figure 5). The

proline content in Giza-111 cultivar and TSU were higher than other cultivars at 25 and 50 mM NaCl treatments, respectively. The proline content showed an increasing trend in all genotypes of chickpea under salt stress especially in tolerant genotypes in keeping with the result of [53]. Proline acts as an intracellular osmotic solute for maintenance of osmotic balance between cytoplasm and vacuole [54]. In addition to its role as an osmolyte and a reservoir of carbon and nitrogen, proline has been shown to protect plants against free radical induced damage and slow utilization of proline for protein synthesis and stimulation of glutamate

conversion to proline during stress may be the possible reason for proline accumulation. Proline is one of the most important osmoprotectant in plants. Under salt stress most plant species exhibit a remarkable increase in their proline content [55,56]. In our experiments we also observed a similar behavior in the seedling of soybean. Supporting findings come from other plants [57, 58], where salt stress resulted in extensive proline accumulation. In support of our observations, recently in rice roots exposed to NaCl stress, a uniform accumulation of proline was shown to be related withincreasing NaCl concentrations [59,51]. Increasing leaf proline content under salinity stress (Figure 5) might be caused by the induction or activation of proline syntheses from glutamate or decrease in its utilization inprotein syntheses or enhancement in protein turnover. Thus, proline may be the major source of energy and nitrogen during immediate post stress metabolism and accumulated proline apparently supplies energy for growth and survival, thereby inducing salinity tolerance [60].

4. Conclusion

In the light of these results, it is concluded that salinity stress inhibit the growth of different soybean cultivars. however, important variability in terms of seedling growth was observed amongst different cultivars of soybean. in general, both tsu and giza-111 seemed to have better potential for salt tolerance compared with other cultivars. the existence of intraspecific genetic variability among soybean cultivars, as shown in this work, might be useful in selective optimal





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