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EVALUATION OF THE SALT TOLERANCE OF SUNFLOWER (*Helianthus annuus* L.) ACCESSIONS USING STRESS TOLERANCE INDICES

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Abstract

Salinity poses a significant abiotic stress challenge that affects plant growth and development, ultimately reducing crop productivity. Owing to the genetic variability present in crop plants, they display a range of responses when faced with salinity stress. This diversity has allowed plant scientists to identify salt-tolerant crop varieties. As a key selection criterion, Plant breeders have been improved the salinity tolerance in different plants through prioritizing seed yield or plant vigor. The evaluation process become feasible and advantageous when crops show clear signs of the salinity tolerance at the cellular level, entire plant, or tissue levels. Salinity varies in the field; therefore, testing plants in a controlled environment with consistent saline conditions is reliable and effective. The hydroponic culture (soilless culture) method is frequently used to study how salinity affects crop plants, allowing researchers to observe the impacts of nutrient deficiencies and toxicities. In this study, 80 sunflower accessions were evaluated in a hydroponic culture for salinity tolerance. Statistical data were collected for the seedling parameters root and shoot lengths, fresh root and shoot weights, dry root and shoot weight, dry root and shoot weight, sodium potassium ratio stress indices. The 10 tolerant (A-18, A-37, A-78, A-35, A-13, A-10, A-30, A-58, A-6, A-64) and four sensitive accessions (A-28, A-80, A-73 and A-29) were carefully chosen based on computed indices from principal component analysis (PCA). In a controlled experimental assay, this study could be useful for comparing salinity indices and identifying salinity-tolerant sunflower accessions for future breeding initiatives.

Key Points: Salinity, Sunflower, Hydroponics, Genetic Variability, PCA.

INTRODUCTION

An important goal that needs to be accomplished by 2050 to guarantee food security is to produce 70 percent higher yield of the food crop to feed extra 2.4 billion human population (Bahar *et al*., 2020). The salinity is an important abiotic stress to crop plants that significantly reduces production in both rainfed and irrigated environments. This reduced crop productivity, resulted by salt affected soils and it can resolve through increasing the salt tolerant plants varieties. Leaching of salts from cultivated soils or cultivating salt tolerant crop plants can help reclaim saline soils.

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Worldwide, upto 800 mha of land are wasted by salt, which accounts for 6 percent of the total soil area. Sunflowers are moderately salt-tolerant (Li *et al*. 2020). Sunflower indicates certain genetic variation in succeeding generations (Riaz *et al*., 2020). Sunflower is a crucial oilseed crop in the global economy, is widely grown for its oil-rich seeds. The oil contents vary from 38 to 50%, commonly used in food, feed and biodiesel (Petraru *et al* 2021; Miladinovic 2019). Seeds of sunflower comprised phenolic compounds, minerals, protein and fibers (Petraru *et al*. 2021).

Sunflower is cultivated in Pakistan, on a small area, the production of edible oil is in deficit range, domestic production of the total oil is covered by only 7% (Economic survey of Pak 2023-24). Increase in the yield of domestic oil production of sunflower is critical, can remain accomplished through expanding cultivated land. This requires utilization of saline soil and development of techniques to improve sunflower salinity tolerance, thereby increasing production under such challenging conditions (Sala *et al*., 2012).

Plant's ability to tolerate salt in soil is a complex trait that is not easily measured by just one characteristic (Li.W *et al*., 2020). Excessive sulfate or sodium chloride in the soil can cause salinity issues (Sima *et al*., 2013). Sodium, a key ion, gets absorbed quickly by plant roots and can lead to toxicity (Subbarao *et al*, 2003). Salinity affects seed germination, growth, and development; as a result, germination rate alone cannot appropriately evaluate the tolerance of sunflower seeds, and other related traits should also be considered when evaluating a plant's response to salinity (Wenhui *et al*, 2020). Salt stress reduces leaf area and dry matter accumulation, and roots and leaves are more salt-sensitive than stems in sunflower (Rivelli *et al*., 2010).

Due to increased Na^+ accumulation and a decrease in the K^+/Na^+ ratio, plant development and yield are reduced (Akram and McNilley, 2013; Haq *et al*., 2013). The germination and growth of hybrids of sunflower (HYSUN 33, DK-3915, HU-777, Super-25, CRN-1435) significantly reduced as soil salinity levels increased (Hafeez, 2017). With more ion accumulation in the plant parts like older leaves and roots, resulted reduction in growth due to specific toxic effects of chloride ions in sunflower (Rivelli *et al*., 2010). Through carrier proteins, ions are entered in the plant cells, which are found on cell membranes. These proteins require ATP and pyrophosphates. Excessive sodium chloride intake reduces the germination of the plant, growth and development of the plant cellular organization (Munns *et al*., 2005; Zhu, 2003).

High level of sodium potassium ratio, calcium sodium ratio maintains turgor pressure in crop plants, may be used in the process of salt tolerant plant selection (Ashraf and Haris, 2004; Shirazi *et al*., 2011). Salinity causes nutritional and hormonal imbalances in sunflower plants, impairing crop productivity, growth and development (Geneti. Takele. Zike, 2019); Shirazi *et al*., 2011). Three mechanisms are involved in salinity tolerance: osmotic-stress tolerance, minimizing Na^+ ion uptake and transport toward the shoots, Na+ exclusion, and tissue tolerance (Munns and Tester, 2008). The maintenance of a low Na^+/K^+ is an important factor which contributes in the salt tolerance (Nguyen.V.L. 2012).

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This can be obtained through combining low amount of sodium flow towards shoot with a high absorption of potassium over sodium ions (Flowers.T.J and T.D.Colmer, 2008). The sunflower wild relatives are moderately salt-tolerant (Serieys, 2010; Baldini and Vannozzi, 2008). It is an important that traits from the wild relatives should be incorporate into the local varieties, to create the salt tolerant plant types, but there are some problems in crossing of wild relatives with local varieties of sunflower due to higher genetic distance, chromosomes pairing become difficult (Mohan and Seetharam, 2005).

It has been investigated that the crossing of wild relatives with local varieties cause severe decline in oil contents (Seiler.G.J, 2007). Therefore, existing germplasm line can be efficiently utilized in breeding program for the production of salt tolerant sunflower hybrids. Due to mode of cross pollination, sunflower has higher magnitude of genetic variability. Plant breeders aim to distinguish salinity-tolerant accessions from existing sunflower accessions. The current study focused on estimating genetic variation, and developing the selection criteria against salinity.

MATERIALS AND METHOD

The sunflower germplasm comprised of 80 lines collected from the Department of Plant Breeding and Genetics at the University of Agriculture, Faisalabad. These lines evaluated at three different salinity levels: 0 mM (considered as control), 120 mM, and 150 mM. The experimental procedure was executed in accordance with a completely randomized design (CRD) under a factorial arrangement.

The experiment was carried out using the wire house facility available at the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Seeds of the 80 sunflower accessions were planted in polythene bags filled with sand, measuring 22.91cm×7.61cm. Each bag contained 6 seed of every line, and four plants in each bag, were maintained until the two-leaf stage. A quantity of 250ml of water applied to each bag on alternate days.

Table 1: Hoagland Nutrient Solution

Reagent	g/L	X1000
$(MgSO_4).7H_2O$	246	246
$Ca(NO_3)_2.H_2O$	236	590
(KNO_3)	101	252.5
(KH_2PO_4)	136	68
Iron (FeEDTA)	37.33	18.66
$(CuSO4 .5H_2O$	0.081	0.041
$(H_2MoO4.H2O)$	0.021	0.012
$(ZnSO_4.7H_2O)$	0.221	0.112
$MnCl_2.4H_2O$	1.812	0.905
(H_3BO_3)	2.861	1.432

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The 3 tubs of 200L volume, used for the preparation of Hoagland solution, using procedure presenting in Table-1 (Hogland.D.R and D. Arnon, 1938). At the two-leaf stage, the seedling of sunflower lines for each replication transplant to the hydroponic solutions. Each tub maintained three replications for every accession under a specific treatment. The three different salinity treatments viz T_0= 0 mM (control), T_1= 120 mM, T_2 = 150 mM, developed through the utilization of NaCl salt.

Subsequent to four days from the transplantation, salt solutions of 40 mM and 50 mM were made and mixed with Hoagland solution. Salt concentrations of 120 mM and 150 mM were made in aliquots of 40 mM and 50 mM on alternate day. The EC of the solutions was obtained through portable electrical conductivity meter (HI-99300), while the concentration of the hydrogen ion (pH) of the concerned solution was adjusted to 7 by using the HCl and NaOH. After twenty-one days of exposure to salinity, three plants from each replication of each accession were up-rooted, and data were collected to the given parameters Root length, shoot length (cm), fresh root weight (g), fresh shoot weight(g), dry root weight(g), dry shoot weight(g) by using an electric balance (Setra BL-410S).

The uprooted root and shoots were placed in separate paper bags, and desiccated for 48 hours at temperature of 65°C (Tanveer-ul-Haq *et al*. 2014), and again calculated the dry weight. The measurement of sodium and potassium ions in tissues of plant followed by (Wolf. 1982). Dried samples of leaves (0.11g) of each accession were placed in separated digestion flasks, and the quantity of 2.5ml H_2SO_4 were added in each flask for overnight at room temperature. 35% H_2O_2 approximately 1ml was added and heated, on the hot plate until the fumes produced at 350 °C.

After that flask were removed from the hot plate to cool before being returned to it after 1ml of H_2O_2 had been added. Until the sample was colorless after cooling, this procedure was repeated. The distill water was mixed with the sample to kept extract volume at 50 ml in the flask. Filtered the extracted material by Whatman filter paper and used for the measuring the sodium and potassium ions. A single-channel flame photometer (Spectronic Camspec Ltd, Model Jenway, PFP-7, UK) was used to analyse K^+ and Na^+ ions with additional purity A range of standards from 5 to 60 mg L^{-1}. The collected data were used to calculate various indices using the following formulas (Ahmad *et al*., 2009; Ashraf *et al*., 2006).

RLSI= (RL of stressed plant / RL of control plant) × 100

SLSI = (SL stressed plant / SL of control plant) × 100

FRWSI = (FRW of stressed plant / FRW of control plant) × 100

FSWSI = FRW of stressed plant / FRW of control plant) × 100

DRWSI= (DRW of stressed plant / DRW of control plant) × 100

DSWSI = (DSW of stressed plant / DSW of control plant) × 100

Na^+ /K^+SI= (Na^+ /K^+ of stressed plant / Na^+ /K^+ of control plant) × 100

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Statistical Analysis: The calculated data was analyzed by analysis of variance (Anova), following Steel *et al*. 1997. Ten salt tolerant and four susceptible genotypes were selected by using the PCA (Principal component analysis) (Yan and Kang, 2011; Gabriel, 1971) on recorded seedling parameters.

RESULT

Genetic Variability

Table 2. The accessions were found significantly different for all salinity indices. Among the treatments, significant differences were recorded except for root fresh weight salinity indices, the interaction between treatment and accessions showed significant differences. Table 2. Shows the range of mean values for T_1 and T_2 stress indices. Treatment-2 stress indices were lower than treatment-1, with the exception of root fresh weight stress indices and Na^+/K^+ stress indices (Graph.1).

Table 2: Mean square value from analysis of variance (ANOVA) for the stress indices in sunflower genotypes under controlled and salt stress condition

Source	DF	RLSI	SLSI	RFWSI	SFWSI	RDWSI	SDWSI	Na^+/K^+SI
Treatment	1	37328.2**	41371.1**	2.54156*	95638.2**	85757.0**	56413.4**	1.017000**
Accessions	79	5.8**	2.3**	3.66505**	14.2**	46.6**	15.8**	141877**
Treat* Accession	79	4.7**	0.5*	0.34410	5.0**	20.2**	8.3**	12200.0**
Error	320	0.8	0.3	0.26502	2.4	5.1	1.2	6463.70

SOV= Source of variation, **T**= Treatment, **A**= Accessions, **E**= Error, **DF**= Degree of freedom, **RLSI**= Root Length stress index, **SLSI**= Shoot Length stress index, **RFWSI**= Root Fresh Weight stress index, **SFWSI**= Shoot Fresh weight stress index, **RDWSI**= Root Dry weight stress index, **SDWSI**= Shoot Dry weight stress index, **Na^+/K^+ SI**= Sodium potassium ratio stress index.

***=** Significance at 0.05 probability level

Table 3: Mean values range of salinity stress indices (%) in Sunflower accessions.

T_1				T_2			
Genotype	Min	Genotype	Max	Genotype	Min	Genotype	Max
23	59.486	79	63.105	80	42.693	13	49.036
73	36.863	37	40.338	31	40.998	18	44.143
5	33.801	38	48.937	73	36.356	53	40.490
54	51.066	67	63.765	5	13.820	52	20.091
80	33.308	37	45.377	54	19.262	51	40.095
18	219.403	73	816.299	29	12.529	18	20.918
31	61.048	79	70.696	18	395.124	9	1179.927

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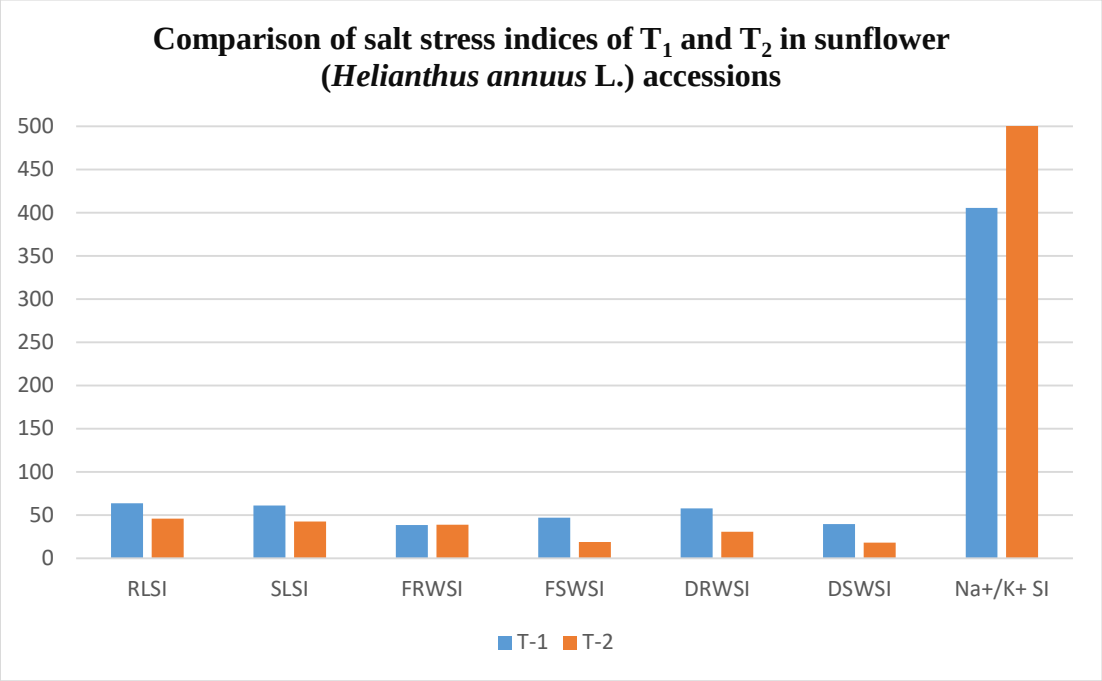
Graph 1: Salinity stress indices of T_1 and T_2 in sunflower accessions



1.1.2. Principal component analysis

Principal component analysis categories genotypes depending on their performance. PCA is also used to categorize genotypes based on desirable yield and yield-related grouped traits. The PCA-1 and PCA-2 biplots are presented in Figures 1 and 2, respectively.

In PCA-1, accessions A-37, A-30, A-63, A-18, A-53, A-78, A-25, A-58, A-13, A-6, A-10, A-11, A-64, A-68, A-2, A-3, A-75, A-38, A-7, and A-52 were presented in Quadrate-1, indicated positive response by all the stress indices towards salinity tolerance for the traits, while A-72, A-45, A-27, A-43, A-68, A-28, A-80, A-19, A-16, A-9, A-54, A-44, A-44, A-46 A-22, A-69, A-71, and A-32 were present in Quadrate -IV, showed all negative responses towards salinity indices, and taken as salt sensitive lines.

Accessions were present in Quadrate-1 considered as salt tolerant A-18, A-13, A-78, A-25, A-3, A-35, A-10, A-15, A-51, A-64, A-33, A-7, A-51, A-58, A-8, A-47, A-11, A-6, A-76, A-36, A-77, A-5, and A-30 , whereas A-73, A-29, A-9, A-28, A-70, A-72, A-39, A-60, A-66, A-34, A-65, and 1 were present in Quadrate IV were salt sensitive accessions. From PCA-1 and PCA-2, lines A-18, A-37, A-78, A-35, A-13, A-10, A-30, A-58, A-6, and A-64 were selected as salt tolerant, while A-73, A-28, A-80, and A-29 were selected as salt sensitive.

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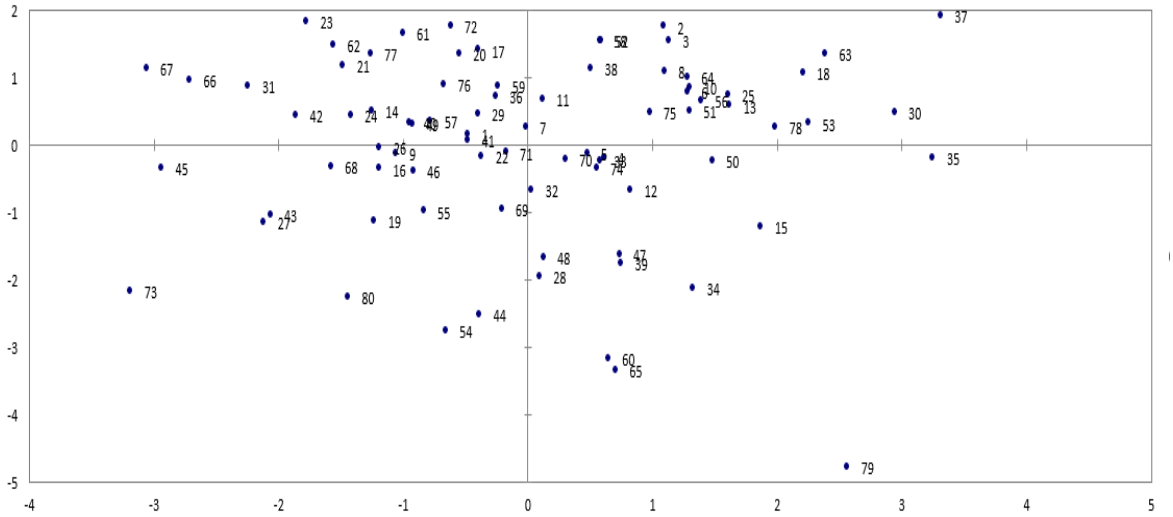

Fig 1: PCA1 of stress indices of T_1 in Sunflower (*Helianthus annuus* L.)

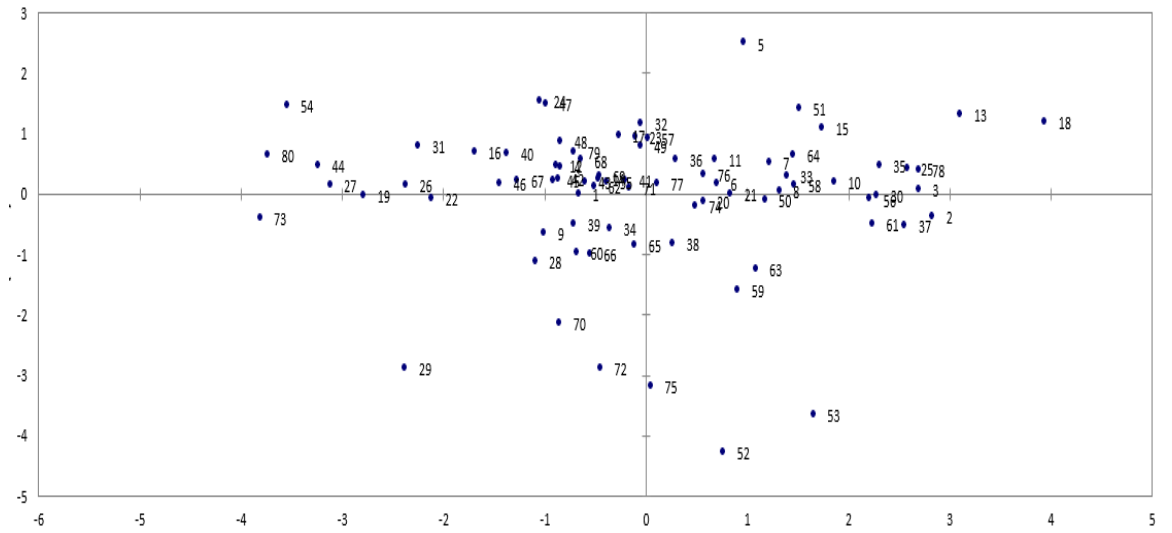

Fig 2: PCA2 of stress indices of T_2 in Sunflower (*Helianthus annuus* L.)

DISCUSSION

Abiotic and biotic stresses are obstacles to agricultural crop growth, development, and yield. Among abiotic stresses, salinity is considered as one of the major limiting factors responsible for the development and production loss of agricultural crops worldwide, especially in dry and semi-arid environments (kaashyap *et al*., 2018). Salinity affects approximately 21-34% of the world's cultivated area, with adverse effects expected to reach 50% by 2050 (Majeed, A and Muhammad.Z., 2019; Machado and Serralheiro 2017).

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Important salts that cause salinity stress are those that contains HCO_3-, Cl-, and SO_4^{-2} (Khan.A, 2009), with sodium chloride being the most abundant and soluble (Munns and Tester, 2008). To tackle the salinity problem, two approaches can be used: (i) chemical amendments to reclaim saline soils, and (ii) to developed the salt tolerant lines. Places in the world where good quality water is not available, or salt affected soils cannot be reacclimated due to the lack of resources.

The intra-specific genetic variation can be exploited by selection under salinity stress conditions (Ashraf, 2004; Ashraf and Harris, 2004). The same findings are obtained when accessions for salinity tolerance are selected under glasshouse/greenhouse circumstances or in the field, during the growing season, selection were made on the basis of salinity tolerant genotypes (Akram and Jamil, 2007). In field conditions salinity occurs in patches so that it is very difficult to identify the salt tolerant accessions, hence it's a more reliable method to evaluate plant under greenhouse condition where salinity is uniformed (Munns and James, 2006).

The hydroponic culture technique is commonly used in the study of salinity's effects on crop plants, it allows for the detection of elemental scarcity and toxicities. It is also useful for evaluating the impact of salinity on plant growth at different stages. It is difficult to measure salt tolerance of crop plants in the field; hence, salinity resistant lines can be identified by cultivating them at various salinity levels using a hydroponic method (Ashraf and Ali, 2008; Ulfat *et al*., 2007). Sunflower accessions with greater variability for salt tolerance can be used for breeding of sunflower for salinity tolerance (Saad *et al*., 2019). This study indicated that selection in this material could be useful for identifying salinity-tolerant and sensitive genotypes.

Determining which traits contribute more significantly to salinity tolerance can be difficult. To make this easier, salt stress indices can be used to determine the contribution of key parameters to salinity tolerance. Stress indices indicated overall biomass production under control conditions and the ability to maintain under stress conditions, promoting the selection of genotypes that perform well under both controlled and stress environments. Choosing the best technique for increasing selection proficiency in breeding operations is a significant task. Principal component analysis can be helpful while dealing with a large number of genotypes and traits (Gabriel, 1981; Yan and Kang, 2011). According to the principal component analysis, accessions A-18, A-37, A-78, A-35, A-13, A-10, A-30, A-58, A-6, A-64 were chosen as salinity tolerant, while A-28, A-80, A-73 and A-29 were chosen as salinity sensitive.

CONCLUSION

The evaluated breeding material contained sufficient genetic variation for all traits. This genetic variability in traits may be used in a breeding programme to improve seedling establishment and also growth rate, resulting in a good crop productivity.

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