IS DROUGHT TOLERANCE IN MAIZE (ZEA MAYSL.) CULTIVARS ATTHE JUVENILE STAGE MAINTAINEDAT THE REPRODUCTIVE STAGE?

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Abstract

Among several abiotic stresses, drought or water scarcity is a major constraint for crop production in many parts of the world. Six maize (Zea mays L.) cultivars; DTC, EV-77, EV-78, EV-79, Faisalabad mays, and 6621 were evaluated for drought tolerance at germination and seedling stages. Distilled deionized water was used as control but uniform drought stress was induced using 3, 6 and 9% of polyethylene glycol-6000 (PEG-6000) which correspond to osmotic potential of -0.0466, -0.0759 and -0.0876 MPa, respectively. PEG influenced the germination and growth of the cultivars in a concentration dependent manner but the highest level of PEG induced more drastic decline for the various attributes studied. The cultivars showed significantly variable responses to different levels of PEG. The result of study clearly suggested variability of characters for drought tolerance among maize cultivars. Based on the pattern of variability for various attributes, 3 groups of cultivars can be classified. The cultivar 6621 had a consistent degree of sensitivity to drought in terms the reduction of various attributes studied. The second group includes DTC which showed a steady tolerance [(germination percentage (GP), energy of emergence (EG), germination rate (GR), root fresh and dry weight (RFW and RDW), shoot fresh and dry weight (SFW and SDW), dry biomass tolerance index (DBTI) and seedling vigor index (SVI)] thus seemed to provide some manifestation of drought tolerance. For the third group of cultivars, pattern of drought tolerance was independent for germination, growth and physiological indices as an incoherent variability of attributes was observed. A similar pattern of variability for a number of characters to simulated water stress in the cultivar DTC served as reliable determinants for drought tolerance in maize. To assess maintenance of degree of drought tolerance selected maize cultivars, a field experiment was also conducted. Kernel vield, 1000- kernel weight (g), number of kernel number/cob, kernel weight/cob (g) was maximally reduced in water stress sensitive cv. 6621 whereas it was maximal in drought tolerant cv DTC. Drought stress at the reproductive stage hindered the floral development and/or fertilization process and thus yield reduction occurs. Overall, selection procedure for selecting drought tolerant maize cultivars was efficient at the germination and seedling growth stages.

Keywords: germination;maize(Zea mays L.); PEG; physiological indices; polyethylene glycol; water stress.

Introduction

Water stress is considered as one of the most devastating environmental stresses worldwide as it has rendered large area of agricultural land unproductive around the globe(Avramova et al., 2015; Huang et al., 2015; Langridge & Reynolds, 2015; Obidiegwu et al., 2015; Zhan et al., 2015). Alterations in rainfall pattern and rising temperature are major causes of drought and have contributed an appreciable decline in crop productivity(Lobell et al., 2011; Langridge & Reynolds, 2015; Obidiegwu et al., 2015). Consequently, considerable agriculture losses occurred because drought-sensitive crops failed to grow under such conditions(Athar & Ashraf, 2009; Huang et al., 2015). It is more likely that increasing population and changing climatic conditions will increase water scarcity, which will cause a further decrease in crop productivity in the world as well as in Pakistan. For example, current trends of climatic changes will increase water scarcity and will reduce maize productivity by 15-30%(Lobell et al., 2014). Therefore, concrete efforts are required to meet the increasing demand for food for heavily populated geographical areas with water scarcity. In order to achieve this target, it is imperative to understand how plants respond and adapt to water stress. The inhibition of plant and root growth due to water stress is the earliestgrowth response, which reduce rate of transpiration thus help in water conservation. However, such effects can reduce the yield up to 60% of maize even if maize plants

do not show leaf wilting (Ribaut et al., 2009). Among different plant adaptive strategies to water stress, drought avoidance is one of the most important drought adaptive strategies that can be used for enhancing crop yield under water stress conditions(Blum, 2011a). This can be achieved in a variety of ways, including adjustment of growth rate and growth pattern of shoot and root(Comas et al., 2013). It is already known that ability of plant for water uptake depends on root system, root structure, and access to water in soil which in turn determine the functionality of plant shoot. Thus, extent of drought avoidance or tolerance in plants can be determined by a number of biometric attributes such as leaf number and structure, root length and branching pattern, leaf waxy layer, leaf rolling etc. (Blum, 2011a; Comas et al., 2013). Since crop sensitivity at the germination growth stage governs overall success of a crop, it is advocated that biometric attributes at the early growth stages can be used as indicator for crop performance at later growth stage or as a selection criteria for improving crop resistance against drought(Lobell et al., 2008; Reynolds & Tuberosa, 2008; Blum, 2011b; Comas et al., 2013). This argument can be supported by the fact that several germination and seedling growth indices are frequently used as predictors to appraise drought tolerance in crop plants(Comas et al., 2013; Ayalew et al., 2014; Shamim et al., 2014; Obidiegwu et al., 2015). The variability in morphological attributes that were associated with tolerance for a target environment can be explored by applying strong selection pressures (Kauser et al., 2006; Huang *et al.*, 2015). Thus, exploitation of inter and intraspecific variation of characteristics for tolerance or avoidance for drought provides an efficient and economic mean of crop selection. As such selected species/cultivars can successfully be grown under drought conditions.

Maize (*Zea mays* L.) is one of the three significant crops of the world following wheat and rice (Ribaut *et al.*, 2009; Cooper *et al.*, 2014; Lobell *et al.*, 2014; Huang *et al.*, 2015).Despite considerable significance of maize as food, forage and oil, a few studies have been focused on the selection of maize germplasmto appraise its drought or water stress tolerance(Avramova *et al.*, 2015). One of the most plausible techniques to simulate uniform drought includes the use of metabolically inactive compound such as Polyethylene glycol (PEG) which has been widely employed by a number of workers to study the effects of water stress in different groups of plants (Ashraf *et al.*, 1996; Kauser *et al.*, 2006; Shamim *et al.*, 2014).

Keeping in view the above aspects, the present study aimed to select maize cultivars that can potentially be grown in a water scarce or drought prone environment. For selection purpose, six cultivars of maize were exposed to varying degree of drought/ water scarce conditions using different concentrations of PEG-6000. Efficiency of selection procedure was evaluated by assessing yield potential of selected maize cultivars for drought tolerance in a field experiment.

Materials and Methods

Seeds of six maize cultivars (DTC, EV-77, EV-78, EV-79, Faisalabad mays, and 6621) were obtained from Ayub Agriculture Research Institute, Faisalabad, Pakistan. The experiment was conducted under laboratory conditions $(32\pm3^{\circ}C)$ at the Institute of Pure and Applied Biology, BahauddinZakariya University Multan, Pakistan. In order to simulate drought stress and the maintenance of uniform osmotic potentials, different concentrations (0 3, 6 and 9%) of polyethylene glycol (PEG₆₀₀₀, Fisher, England)were used which correspond to-0.0466, -0.0759 and -0.0876 MPa osmotic potentials, respectively. Twenty surface sterilized seeds of each maize cultivar were placed on to appropriately labeled plastic trays (30cm×25cm×10cm) on 6 layers of filter paper (Whatman No.1). The experiment was arranged in Complete Randomized manner with three а replicates. Varving levels of water stress was simulated by adding 150 mL of different concentrations of PEG-6000in respective plastic tray. Seed germination was recorded on daily basis till 10 days. Seeds were considered germinated when the emerging radicals and plumules were \cong 0.2 cm in length. Germination percentage was calculated as per cent ratio of germinated seeds out of total seeds. Seed germination rate was calculated as (100/n) (N3/3+N5/5)Where n= total number of seeds, N3= number of seeds germinated on 3rd day, N5= number of seeds germinated on 5^{th} day. Indices of seed germination, seed stress tolerance and seed promptness were also calculated as

Promptness index (PI) = nd2 (1.00) +nd4 (0.75) +nd6 (0.5) +nd8 (0.25); where n is the number of seeds germinated at day d.

Seedling Vigor Index (SVI) was calculated by as:Seedling length (cm) \times germination percentage

Stress tolerance index was calculated following Ashraf *et al.* (2006)as: (Trait of stressed plant/ trait of control plants) x 100

The experiment continued for ten days then fresh weights and length measurements of roots and shoots were taken. Seedling material was oven dried at 70°C for 24 hours then dry weights were taken after complete desiccation.

Assessment of yield potential of selected maize cultivars at various moisture regimes under field conditions: In a field experiment, seeds of selected maize cultivars (DTC, EV-78 and 6621) were sown on row ridges of plots (12 x 12 ft). Row spacing was 24 inch and plant spacing was 9 inch. There are two irrigation treatments control and water stressed; in water stressed treatment irrigation was withheld at the tassel forming stageup to wilting point and leaf rolling stage while the well-watered plants continued to receive irrigation to the field capacity each week till physiological maturity. Fertilizers (urea, DAP and potassium sulphate) were applied as per required. Maize cultivars were kept free of weeds by hoeing to avoid the weed crop competition.Plot area $(12 \times 12 \text{ ft}^2)$ of each treatment was harvested and 20 sub-samples of each maize cultivar were taken for the analysis of varying yield attributes. The following yield components were measured according to standard procedures:

Weight of kernels per cob (g): This was selected at random from the grain lot of twenty cobs of plot area $(12 \times 12 \text{ ft}^2)$ and weighed with the help of electric balance.

Number of kernels per cob: Number of kernels of 20 cobs from each plot area were counted and taken average.

1000-Kernel weight (g): This was taken at random from the grain lot of each plot area and weighed by electric balance.

Grain yield: Grain yield was recorded by weighing the kernels shelled from the cob from the central four rows of each plot area $[12 \times 12 \text{ ft}^2 \text{ or} 3.5676 \times 3.5676\text{ m}^2]$ and converted it into kg ha⁻¹ using the formula:

Grain yield (kg ha⁻¹): = [Grain yield (kg)/harvested area $(3.5676m \times 3.5676m)$] × 10000

Then this mass of kernels/ plot converted into kilograms/ hectare (kg/ha.)

Statistical analysis: Data for all attributes were presented as mean values with<u>+</u>S.E. The data for various morphophysiological attributes were subjected to a Two Way Completely Randomized Analysis of Variance (2WCR ANOVA) using a COSTAT computer package (Cohort Software, Berkeley, California) to elucidate effects of PEG levels as well as to reveal intraspecific variability. The mean values for each factor were then compared to find out Least Significant Difference (LSD) following (Snedecor & Cochran, 1980).

Results

Seed germination of six maize cultivars declined significantly due to PEG_{6000} induced water stress. Maize cultivars also differed significantly under both normal and

PEG-induced water stress conditions (Table 1). Among cultivars, DTC exhibited the maximum germination percentage (95%) at the highest concentration of PEG, whereas cv. 6621 was the lowest in this attribute at the highest level of PEG-induced water stress (Fig. 1). In addition, cvs. EV-77 and EV-78 were intermediate in seed germination percentage (Fig. 1).Rate of seed germination of all maize cultivars reduced significantly ($P \le 0.001$) due to water stress particularly at 9% PEG (Table 1; Fig. 1). Off all maize cultivars, cv. DTC showed highest rate of seed germination at all concentrations of PEG. In contrast, cv. 6621 had the lowest germination rate at all levels of PEGinduced water stress (Fig. 1). Remainder cultivars were intermediate in this attribute at all levels of water stress (Fig. 1). Simulated water stress reduced the energy of emergence of all maize genotypes particularly at the highest level of PEG (9%). Maize cultivars significantly varied (P≤0.001) in energy of emergence at all levels of PEG-induced water stress (Table 1).

Imposition of water stress by PEG caused a drastic reduction in shoot fresh and dry biomass of seedlings of all maize cultivars. Among the cultivars, DTC was the highest in fresh and dry weight while the lowest was cv. 6621 at all level of water stress (Fig. 2). Moreover, cv. EV-78 was intermediated in this morphometric attribute at all levels of PEG-induced water stress. A significant reduction in root fresh and dry biomass of maize cultivars was noticed due to PEG-induced water stress. The adverse effect of PEGinduced water stress on root fresh and dry biomass of all maize cultivars was maximal at 9% PEG. In addition, the reducing effect of PEG-induced water stress was minimal on the root fresh and dry weight of cv. DTC as compared to all other cultivars of maize (Fig. 2). Shoot and root lengths of all maize seedlings were also reduced with increasing level of PEG in the growth medium (Table 1; Fig. 2). Cultivars also differed significantly in these growth attribute ($P \le 0.001$). Among cultivars, 6621 was the lowest in having in shoot and root length at the highest level of PEG-induced water stress (9%), whereas cv. DTC was the highest in these attributes (Fig. 2).

Since genotypic responses to varying levels of PEG in the growth medium in all these biometric markers varied significantly, these attributes were further assessed based on tolerance indices. Germination tolerance index (GTI) decreased progressively in maize cultivars as the concentration of PEG increased in the growth medium. The cultivars varied in germination tolerance index at varying concentration of PEG (Fig. 3). Among cultivars, highest GTI was recorded in EV-78 and EV -79, whereas cv. 6621 was the lowest in this attribute at the 9% PEG-induced water stress (Fig3). Seedling vigor index of all maize genotypes was severely declined due to PEG-induced water stress. All cultivars had shown significant (P≤0.001) variation in SVI under PEG-induced water stress (Table1). Off all maize genotypes, DTC had greater seedling vigor index at all levels of water stress induced by PEG (Fig. 3), whereas the reverse was true for cv.6621 at all levels of water stress. In addition, all remaining maize genotypes were intermediated in this attribute. Among cultivars, cv. DTC exhibited greater EG at all concentrations of PEG, whereas cvs. EV-77 and 6621 were the lowest in this seed

germination attribute at the highest level of PEG-induced water stress (Fig. 3).It was found that dry biomass stress tolerance index (DBTI) was the lowest for cv. 6621 at 6% and 9% PEG concentration in the growth medium, whereas the all other cultivars were similar in this attribute at all levels of water stress (Table1; Fig. 3). At low and moderate level of PEG-induced water stress, cv. EV-78 was the lowest in plant height stress tolerance index (PHTI) as compared to all maize cultivars, whereas at the highest level of water stress cvs. EV-78 and 6621 were the lowest in this attribute. In contrast, root length tolerance index was the highest in cv. Faisalabad mays followed by cv. EV-79 at 9% PEG level (Fig. 3), while the remainder cultivars were similar in RLTI at the highest level of PEG-induced water stress.



Fig 1. Germination percentage (GP), energy of emergence (EG), germination rate (GR) of six Zea mays L. cultivars grown at varying PEG6000 concentrations (0, 3, 6 and 9%) for 10 days.

Drought stress at the reproductive stage especially during tassel formation in maize (Zea mays L.) hindered the fertilization process because pollens remain immature and thus yield reduction occurs. In the present study, kernel vield (kg/ha) and kernel vield components of maize cultivars (DTC, EV-78 and 6621) such as 1000- kernel weight (g), number of kernel number/cob, kernel weight/cob (g), were significantly ($p \le 0.001$) reduced due water stress (Table 1; Fig. 4). Maize cultivars (DTC, EV-78 and 6621) were also differed significantly in these attributes. Kernel yield was maximally reduced in water stress sensitive cultivar 6621 and relative yield reduction (RYR) in this water sensitive maize cultivar was 75%. In contrast, kernel yield was maximal in water stress tolerant cv. DTC in which relative yield reduction was 48%. In addition, cv. EV-78 remained intermediate in kernel yield and showed 64% relative yield reduction due to water stress (Fig. 4). To assess contribution of different yield components in degree of water stress sensitivity, different yield components were assessed. Thousand-kernel weight (1000-kernel weight) is a significant yield contributing factor, which plays a decisive role in showing the potential of a cultivar under stress environment. Data regarding the 1000-kernel weight (g) revealed that drought cycles significantly reduced 1000-kernal weight in all maize cultivars (Table 1). However, the reducing effect of water stress on 1000-kernal weight (kernel size) was minimal in cv. DTC and water stressed plants of cv. 6621 had the lowest 1000-kernal weight (Fig. 4). Number of kernels per cob is an important materialistic character which contributes towards the final grain yield. Kernel number per cob significantly ($p \le 0.001$) decreased in maize cultivars (DTC, EV-78 and 6621) due to the imposition of drought. The maximum reduction in number of kernels per cob was found in cv. 6621, whereas the reverse was true for cv. DTC. Cultivar EV-78 remained intermediate in this yield attribute under water stress (Table 1; Fig. 4; Colour Plate 1).

Table 1. Mean square values from ANOVA for germination percentage (GP), energy of emergence (EG) and germination rate (GR) of six maize cultivars grown at varying PEG-6000concentrations for 10 days.

Source of variance	df	Germination Percentage		Energy of Emergence		Germinatio n Rate
cultivars (cvs.)	5	833.1***		1.572***		227.1***
PEG conc.	3	1017.9***		6.182***		302.5***
cvs. × PEG conc.	15	52.93***		0.197ns		6.219ns
Error	48	12.15		0.122		3.462
		Shoot Fresh	Shoot Dry	Root Fresh	<mark>Root</mark> Dry weigh Shoot	Root
Source of variance	df	Weight	weight	Weight	t Length	Length
cultivars (cvs.)	5	27284.7***	471.9***	18459***	<mark>282.8</mark> *** 18.85*** 257.8	51.47***
PEG conc.	3	38702.2***	230.6***	27333***	*** 29.34*** 5.654	213.8***
cvs. × PEG conc.	15	969.2ns	7.491ns	1222*	ns 2.36***	7.529***
Error	48	971.8	8.597	588.8	10.66 0.285	2.162
Source of variance	df	Germination Tolerance Index	Root Length Tolerance Index		Seedling Vigour Index	Dry Biomass Tolerance Index
cultivars (cvs.)	5	740.8***	543.3***	1110***	1688870***	409**
PEG conc.	3	3576.2***	4673.3***	2801***	4384007***	1721***
cvs. × PEG conc.	15	36.95ns	143.2ns	97.21ns	128822**	68.7ns
Error	48	112.1	79.4	0.6111	40822.7	82.74
Source of variation	df	Kernel weight/cob	Kernel yield		1000 kerne weight	l <mark>Kernel</mark> number/cob
Drought cycles	1	51543***	103087***	<mark>3482**</mark>	** 510125	***
cvs	2	956***	1912***	<mark>801***</mark>		<mark>***</mark>
Drought cycles*cvs	2	718**	1437***	<mark>238***</mark>	[•] 15191*	<mark>***</mark>
Error	234	116	113	12.70	<mark>1913</mark>	
Total	239	······································				

ns=non-significant; *,**,*** significant at 0.05,0.01 and 0.001 probability levels, respectively.



Fig.2. Cobs of selected maize cultivars showing yield potential of maize cultivars under normal and water stress conditions under field conditions.



Fig 3. Germination, dry matter, plant height, root length and seedling vigor indices of six maize (Zea mays L.) cultivars grown at varying PEG6000 concentrations (0, 3, 6 and 9%) for 10 days.



Fig. 4. Effect of four drought cycles on yield and yield components of maize cultivars (DTC, EV-78 and 6621) in a field experiment conducted during January to April, 2013.

Discussion

The establishment of a crop in a hostile environment largely depends on successful germination and early seedling establishment which are key stages in the life cycle of a plant. Therefore, it becomes imperative to develop efficient screening methods and suitable recurrent selection criteria at early establishment phases to get optimum yield (Ayalew *et al.*, 2014; Cooper *et al.*, 2014). For this study we have considered germination and growth attributes as well as physiological indices to assess drought tolerance in maize cultivars.PEG has been utilized to induced water deficit environment as it creates uniform osmotic potential and reflects the type of stress imposed by a drying soil.

Seed germination is limited by increasing strength of drought because water is crucially required for imbibitions, subsequent cell division and development of embryonic axes; radicle and plumule(Athar & Ashraf, 2009; Shamim et al., 2014; Avramova et al., 2015). Increasing levels of water deficit induced by ascending concentration of PEG differentially influenced germination percentage, germination rate and energy of emergence in maize cultivars (Fig. 1).Greater susceptibility of germination attributes in cultivars was observed to intensive drought condition induced by the highest level of PEG which hampered the availability of water owing to decline in osmotic potential. Several other workers have also reported similar findings where increasing concentrations of PEG caused more drastic effects on germination(Kauser et al., 2006; Waseem et al., 2006; Ali et al., 2007; Ashraf et al., 2007; Shamim et al., 2014). However, such adverse impact on seed germination and seedling growth varies among cultivars of same species and it depends on genetic potential of crop cultivar (Ashraf et al., 2006; Shamim et al., 2014). For example, in the present study, cultivars DTC and EV-79 had better germination and greater root length in DTC under severe moisture deficit. It is suggested that longer roots might helped in water absorption from the growth medium and thus supported in biomass accumulation under water deficit conditions. Thus, the variability among genotypes under water stress condition as observed earlier (Ashraf et al., 2006; Puangbut et al., 2010; Shamim et al., 2014) indicates that drought tolerance can be attained by the alteration of only those growth attributes which are advantageous for stress hit environment but are under genetic control. Moreover, application of strong selection pressure and growth tolerance indices can help in exploring innate genetic potential of diverse germplasm. For example, root length tolerance index, shoot length tolerance index and use of other similar indices has widely been reported in the literature (Ashraf et al., 2006; Zhang et al., 2010).

Cultivar DTC had higher for seedling vigor and dry biomass tolerance indices, whereas root length tolerance index (RLTI) was higher in Faisalabad mays. Similarly, cultivar EV-77 was higher in shoot length tolerance index (SLTI). Thus, differential responses of the cultivars were noticed for various tolerances indices and it is difficult to discriminate cultivars based on single tolerance index. It pertinent to mention here that sensitivity of SVI is greater than other tolerance indices. Thus, based on GTI and SVI, cultivars were discriminated for water stress tolerance as has been observed in some of earlier studies (Huang *et al.*, 2015; Zhan *et al.*, 2015).

Under harsh set of environmental conditions, germination success alone cannot guarantee successful seedling establishment and vice versa. Plant responses to water stress varied significantly at various growth and developmental stages depending upon the severity and duration of stress (Athar & Ashraf, 2009; Ayalew et al., 2014). In addition to this, the variation in the degree of drought tolerance is structured by the species specific morpho-physiological response during the different growth stages (Blum, 2011a; Shamim et al., 2014; Avramova et al., 2015). In the present study, degree of water stress tolerance varied in some of maize cultivars but cv. DTC remained water stress tolerant in terms of biomass and yield production. Similarly, cv. 6621 remained water stress sensitive at the adult vegetative and reproductive growth stages. To affirm this, a field experiment was also conducted to assess yield potential in selected maize cultivars, as yield potential is the foremost attribute in assessing drought tolerance. As described earlier, various morphometric attributes and physiological processes are directly translated in yield. However, extent of reduction in yield due to drought stress depends on duration and intensity of drought stress, plant developmental stage at which plant experiences drought stress and plant genetic potential to cope with drought stress. However, the most damaging impact of drought stress on yield potential of crop plants occurs when crop plants experiences water deficit during reproductive phase as is recently observed in chickpea (Pushpavalli et al., 2015) and tomato (Shamim et al., 2014; Shamim et al., 2015). From the results of the present study, water stress imposed at the vegetative or at the reproductive growth stage caused a lesser decrease in kernel vield in drought tolerant cv DTC than in other cultivars. Drought stress reduced both kernel number and weight, but the kernel number was most affected due to drought. These results can be explained in view of the fact that maize is more sensitive to drought stress at the reproductive than other cereals because anthers and the silks are separated at a distance of about one meter and there is more chance of exposure of pollens and stigmas with their surroundings(Cooper et al., 2014; Lobell et al., 2014). Moreover, adverse effects of water stress on dry matter partitioning to reproductive tissues also resulted in development of lesser number of ovules with subsequent fertilization(Edreira & Otegui, 2013). Our results also showed that kernel number per cob decreased due to drought stress and maximum reduction in this yield attribute was found in cv. 6621. Although we did not measure degree of floral abortion, metabolic activity of developing kernels, the conceptual frame work allow us to speculate that there was a larger decrease in kernel set

(Plate. 1) due to greater sensitivity of fertilization of flowers to develop kernels or ovule development in water stress sensitive cultivar 6621 than the other maize cultivars, whereas in water stress tolerant cv DTC there was a lesser decrease in number of kernel set with better supply of assimilates from the source (photosynthetic tissue) to sink(Edreira & Otegui, 2013). Thus it is suggested that differential yield potential of maize genotypes examined in the present study due to one of the above mentioned reasons or combination of these factors.

It is concluded that degree of drought tolerance was maintained at different growth stages in six maize cultivars and selection procedure at the germination and seedling stage was effective in discriminating cultivars. In addition, reliable selection criteria are based on the number of correlated characters rather than a choice of few attributes. Therefore, a coherent pattern of variability among characters will result in more tolerant genotypes at later stages with more economic yield.

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