

Evaluation of end-of-season drought stress tolerance in some rice genotypes, north of Iran

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ABSTRACT

Drought stress has been well documented as an effective parameter in reducing crop production. Hence, developing and releasing new genotypes adapted to water deficit conditions can be a constructive way to overcome to unsuitable environmental conditions. Canonical discriminant analysis (CDA) was used in combination with cluster analysis to evaluate the response of 14 rice genotypes using the drought stress tolerance indices under two conditions of irrigation and discontinued irrigation at a specific time. The CDA results revealed that the first canonical variable was under the influence of high coefficients for the stress tolerance index (STI), harmonic mean (HM), mean production (MP), geometric mean production (GMP), yield in normal conditions (Yp), and yield in stress condition (Ys). The canonical coefficients of the stress sensitivity index (SSI) and tolerance index (TOL) were significant in the second canonical variable. Accordingly, the first canonical variable distinguished the genotypes based on yield potential and stress tolerance, while the second one segregated the susceptible and tolerant genotypes. The scatter plot chart of two significant canonical variables distinguished three groups, and all pairs of Mahalanobis distances between groups were significant. The second group was recognized as the best group, since its genotypes had the maximum value for the first canonical variable, while most of these genotypes had low values for the second one. Therefore, the genotypes of this group (831, Fajr, Sepidrood and Sahel) can be selected as suitable genotypes for stress and non-stress conditions.

Keywords: Canonical discriminant analysis, Drought tolerance indices, Rice, Stress.

INTRODUCTION

One of the major abiotic stresses influencing crop is water deficit. This effect is more pronounced in rice that mainly completes its life cycle under water condition. Water stress is, therefore, a major abiotic constraint for rice productivity (Kumbhar *et al.* 2015). Diminishing water supplies for agriculture is an increasing trend. This necessitates the search for drought adaptation in rice. Screening for rice varieties tolerant to water stress is seen as an important step in sustaining further development of rice production (Pandey & Shukla 2015). Several stress indices have been proposed for the screening drought stress compatibility. These indices consider the relationships between traits in stress and non-stress environments. According to Rosielle & Hamblin (1981), Fernandez (1992), and Sareen *et al.* (2012), these indices can be divided into two groups. The first group represents sensitivity indices including tolerance index (TOL) and stress sensitivity index (SSI) which allow for a possible differentiation between tolerant and sensitive genotypes and often exhibit a negative correlation with yield.

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TOL was first proposed by Rosielle & Hamblin (1981) and indicates the difference in yield under normal (Yp) and stress (Ys) conditions. High TOL values reflect stress susceptibility. On the other hand, Fischer & Maurer (1978) proposed SSI. Low levels of SSI indicate higher tolerance to stress. The second group are tolerance indices including mean production (MP), geometric mean production (GMP), and especially stress tolerance index (STI) which can identify high yielding and tolerant genotypes as well as exhibiting a positive relationship with yield. The MP and GMP indices are the mean and the geometric mean of yield in stress and non-stress environments respectively (Rosielle & Hamblin 1981). STI was proposed by Fernandez (1992). High levels of STI indicate high tolerance to stress and a greater yield potential. The STI, GMP, and MP are the most recommended indices for identifying high yielding genotypes in both stress and non-stress environments. However, tolerance and susceptibility indices are not ideal for recognizing genotypes with high yield and stress tolerance under both stress and non-stress conditions (Thiry *et al.* 2016).

The combination of these two groups of stress indices (tolerance and sensitivity) has been proposed as a useful scale for improving drought stress tolerance (Ramirez-Vallejo & Kelly 1998). Accordingly, in this study, canonical discriminant analysis (CDA) was used to identify the superior genotypes based on a combination of calculated stress indices. As a multivariate statistical method, CDA performs the differentiation of groups by considering all the variables simultaneously. The CDA can separate intergroup from intragroup effects by maximizing the distinction between groups (Riggs 1973). After determining the interdisciplinary variation, the Mahalanobis distance statistic (D2) can be used as an index of the difference between the groups (Loos 1993). Data from CDA can then be used to group the genotypes into smaller subgroups which are similar to each other (Loos 1993). Abarshahr *et al.* (2011) reported that mean harmonic (HM), STI, GMP and MP can be used as the best indices to introduce high grain yielding and also tolerant cultivars in rice breeding programs. Khan & Dahrr (2016) used stress tolerance indices to identify drought-tolerant genotypes in rice. They found that SSI, TOL, and YSI could be used to screen sensitive and tolerant genotypes under drought stress at reproductive stage.

Rajiv *et al.* (2010) employed CDA to distinguish rice genotypes based on tolerance and stress susceptibility. They observed that CDA could be used as a potential screening tool for identification. Notably, the tolerant genotypes were used in the early stages of rice growth. Riggs (1973) used CDA to select among a population of spring barley, suggesting that this method could be used as an alternative to the selection index, when it is difficult to allocate economic weights to traits. Rascio *et al.* (2012) used CDA to identify physiological traits capable of differentiating between durum wheat genotypes for compatibility with semi-arid environments. They concluded that a function based on 13 morpho-physiological genotypes differentiated them into three groups: adaptable, semi-adaptable, and non-adaptable. The purpose of this study was to simultaneously use the susceptibility and stress tolerance indices for identification of water stress-tolerant genotypes and high yielding genotypes under field conditions.

MATERIALS AND METHODS

In this study, the response of 14 rice genotypes to drought stress was evaluated using drought stress indices as a split plot experiment based on a randomized complete block design with three replications between 2016 and 2017 at Research Institute of Rice, Rasht, Iran. The genotypes in this study included L203, L416, L831 and L841 lines as well as Dorfak, Fajr, Sepidrud, Khazar, Sahel, Neda, Sa13, Hashemi, Alikazemi and Sangjo cultivars. In both experiments, plant water requirements in seedlings and vegetative growth stages were completely provided (flooding with 5-cm high). When the genotypes reached the flowering stage, the water required for stress conditions was provided in the form of periodic irrigation every 11 days. The area of each experimental plot and also the seedling spacing were 9 m² and 25 × 25 cm respectively. Based on a soil test, 100 kg ha⁻¹ triple superphosphate, 100 kg ha⁻¹ potassium sulfate, and 100 kg ha⁻¹ urea were added as basic fertilizers with the first plough. Similarly, 100 kg ha⁻¹ urea was added in two stages before the first and second weed controls. The measurements were performed taking into account the marginal effects for grain yield (g/plant). After examining the normality of the residual variables, analysis of variance (Gomez & Gomez 1984) was used by SAS 9.0 (SAS 2002) to identify significant differences between the generations for grain yield. The drought stress tolerance indices were calculated using the following relations:

$$SSI=\left[1-\left(Ys-Yp\right)\right]/\left[1-\left(\bar{Ys}-\bar{Yp}\right)\right]$$

Caspian J. Environ. Sci. Vol. 19 No. 2 pp. 341~352 DOI: ©Copyright by University of Guilan, Printed in I.R. Iran (Fischer & Maurer 1978)

$$MP = (Yp + Ys)/2$$
(Hossain *et al.* 1990)

$$TOL = Yp - Ys$$
(Rosielle & Hamblin 1981)

$$STI = (Yp \times Ys)/(\bar{Yp})^2$$
(Fernandez 1992)

$$GMP = (Yp \times Ys)^{\frac{1}{2}}$$
(Fernandez 1992)

$$HM = 2(Yp \times Ys)/(Yp + Ys)$$
(Schneider *et al.* 1997)

where, Ys is grain yield under drought stress condition, Yp represents the grain yield under non-stress condition, \bar{Ys} shows the yield mean under drought stress condition, \bar{Yp} denotes the yield mean under non-stress condition, and $1 - (\bar{Ys} - \bar{Yp})$ is the severity of stress.

CDA is a combination of principal component analysis (PCA) and canonical correlation analysis (CCA; Vaylay & van Santen 2002). Linear combinations of the main variables was created from PCA for justifying the greatest diversity. Based on canonical correlations, a linear relationship is established between predictive variables and standard measures (Dillon & Goldstein 1984). Thereafter, according to a categorical variable and a few quantitative variables, the CDA of canonical variables is obtained. The canonical variables are linear constants of the quantitative principal dimensions with the maximum possible multiple correlations with each group. They are also the best aggregate of intergroup variations. Linear composition coefficients are canonical coefficients or canonical weights. The canonical variables are non-continuous even if the measured traits are highly correlated. In CDA, the distinction between groups is based on the correlation between the independent variables and their relationships with the dependent variable (classification variable; Vaylay & van Santen 2002). Maximum multiple correlation is called the first canonical correlation. The second one is obtained by finding a nonlinearlinear composition accompanied by the first canonical variable with the maximum degree of multiple correlations among the groups. The process of extracting canonical variables can be repeated as long as the number of these variables is equivalent to the number of main variables or the number of classes, minus one (whichever is less). In CDA, as a multivariate statistical method, all variables are considered simultaneously in the differentiation of groups. CDA can separate intergroup effects from intra-group effects by maximizing differentiation between groups when tested against intra-group variations (Riggs 1973). After determining the inter-group variation, the Mahalanobis distances can be used as an indicator of the difference between the groups (Loss 1993). The difference between the values of the center of the two groups shows the Mahalanobis, which is calculated as follow:

$$D^{2} = (\bar{x}_{1} - \bar{x}_{2})' S^{-1} (\bar{x}_{1} - \bar{x}_{2})$$

where, S^{-1} is the inverse of the variance matrix of the covariance sample which is aggregated; the corresponding vectors are measurements for groups x₁ and x₂ (Dillon & Goldstein 1984).

Cluster analysis and CDA were performed using SPSS version 16.0 (SPSS 2007). In order to perform cluster analysis, at first, the coefficient of distance between genotypes was calculated using Euclidean distance square method and then the dendrogram was mapped by Ward method. The dendrogram cutting point was determined based on the results of the discriminant function. After cutting the dendrogram, the primary grouping accuracy was obtained from cluster analysis by evaluating the discriminant function. CDA and drawing scatter chart of groups were performed based on canonical variables via SAS 9.0 software (SAS 2002). The CANDISC procedure was used in CDA.

RESULTS AND DISCUSSION

Analysis of variance of grain yield of genotypes under two irrigated and discontinued irrigated conditions since the onset of panicles revealed that there was a significant difference between genotypes for grain yield. Further, the significance of the interaction between genotype \times irrigation treatments indicated a different reaction of genotypes to irrigated and stressed conditions (Table 1).

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Source of variation	DF	Mean squares
Year	1	22846.0 ^{ns}
Block (Year)	4	208333.8**
Water regime [W]	1	192867960.2**
Year * W	1	1162512.7**
W * Block (Year)	4	24569.3
Genotype [G]	13	5798748.2**
G * W	13	1069039.4**
Year * G	13	189211.9**
Year * W * G	13	121602.7**
Error	104	23329.6
CV	4.07	

 Table 1. Combined analysis of variance for grain yield of rice genotypes under two water regimes in two growing seasons.

ns, *, **: Not significant, significant at 0.05 and at 0.01 probability levels, respectively.

In each year, cluster analysis of genotypes based on stress tolerance indices (STI, HM, MP, GMP, Yp, Ys, TOL, and SSI) divided them into three groups (Figs. 1-2), where groups 1-3 consisted of 6, 4, and 4 genotypes respectively. Bahrami *et al.* (2014) using cluster analysis based on drought tolerance indices, were able to divide the genotypes of safflower into tolerant and susceptible groups.





Fig. 2. Dendrogram of the cluster analysis of genotypes (2nd Year).

Table 2. Results of discriminant analysis for verifying the accuracy of grouping by the cluster analysis (1st Year). Predicted Group Membe rchir

Groups		r reucted Group Memoersmp					
Groups		1	2	2	Total		
	1	6	0	0	6		
Count	2	0	4	0	4		
	3	0	0	4	4		
	1	83.3	16.7	0	100.0		
%	2	0	100.0	0	100.0		
	3	0	0	100.0	100.0		
The correct grouping		92.9%.					

The correct grouping

Table 3. Results of discriminant analysis for verifying the accuracy of grouping by the cluster analysis (2nd Year).

		Predicted Group N	Aembersh	ip	
Groups		1	2	2	Total
	1	6	0	0	6
Count	2	0	4	0	4
	3	0	0	4	4
	1	100.0	0	0	100.0
%	2	0	100.0	0	100.0
	3	0	0	100.0	100.0

The correct grouping

100%.

Table 4. Multivariate statistics for testing the assumption of the equality of the mean vectors of the groups (1st Year).

Statistic	Value	F Value	Num DF	Den DF	Pr>F
Wilks' Lambda	0.00	55.16	16	8	<.0001
Pillai's Trace	1.95	26.46	16	10	<.0001
Hotelling-Lawley Trace	569.92	142.48	16	4	0.0001
Roy's Greatest Root	548.36	342.73	8	5	<.0001

Table 5. Multivariate statistics for testing the assumption of the equality of the mean vectors of the groups (2nd Year).

Statistic	Value	F Value	Num DF	Den DF	Pr>F
Wilks' Lambda	0.001	14.38	16	8	0.0003
Pillai's Trace	1.83	6.95	16	10	0.0018
Hotelling-Lawley Trace	144.11	36.03	16	4	0.0017
Roy's Greatest Root	138.77	86.74	8	5	<.0001

CDA, through two or more observation groups containing several quantitative variables, generated linear combinations of variables involving the highest possible correlation with the groups. The canonical correlations are presented in Tables 6 - 7. A likelihood ratio test has also been provided for the assumption that the current canonical correlations and all the smaller correlations are equal to zero. An approximation of F (Rao 2009) was used to test these coefficients.

Table 6. Canonical correlations and eigen values in CDA (1^{st} Year).						
Canonical variable	Canonical correlation	Eigen value	Cumulative percentage	Likelihood ratio	Approximate F value	
		-				
1	0.999	548.370	0.962	0.00	55.16	
2	0.977	21.560	1.000	0.044	15.40	

Table 7. Canonical correlations and eigen values in CDA (2 nd Year)						
Canonical variable	Canonical correlation	Eigen value	Cumulative percentage	Likelihood ratio	Approximate F value	
1	0.996	138.77	0.963	0.001	14.38	
2	0.917	5.338	1.000	0.157	3.81	

The canonical loads, also called canonical structures (Table 8), constitute a simple linear correlation between the principal independent variables and the canonical ones. Thus, the canonical loads reflect the common variations between the measured variables and the canonical one which can be interpreted once assessing the relative contribution of each variable to each of the canonical ones (Cruz-Castillo *et al.* 1994). The first canonical variable was affected by high loads for STI, HM, MP, GMP, Yp, and Ys. According to Fernandez (1992), the canonical loads of SSI and TOL were significant in the second canonical variable (Table 8).

Selection based on MP and GMP leads to the screening of high yielding genotypes in both stressed and nonstressed conditions. STI is able to distinguish genotypes from other genotypes with high yields in both nonstressed and stress-resistant conditions.

The higher value of TOL indicates more susceptibility to stress. Hence, the low value of this index is suitable for selecting tolerant genotypes. Although low SSI genotypes are considered as stress tolerant since they show lower yields under stress conditions than non-stress conditions, SSIs between genotypes with a potential for stress tolerance and low-yielding genotypes are not distinguished (Ramirez-Vallejo & Kelly 1998).

Therefore, the first canonical variable can distinguish genotypes based on the yield potential and stress tolerance. Given the positive and high values of this variable, the selected genotypes will have a high yield in both stressed and non-stressed conditions. The second canonical variable distinguishes the tolerant genotypes from susceptible ones. Therefore, the selected genotypes for the high values of the first canonical variable and for the low magnitude of the second variable are suitable for both stress and non-stressed environments. The mean of the groups for canonical variables is presented in Table 9.

	1 st Year		2 nd Year	
Variable	First canonical variable	Second canonical variable	First canonical variable	Second canonical variable
Ys	0.937	0.285	0.946	-0.190
Yp	0.786	-0.406	0.822	0.361
MP	0.953	-0.139	0.934	0.089
GMP	0.984	-0.023	0.948	0.027
HM	0.987	0.084	0.955	-0.027
STI	0.980	0.000	0.636	-0.215
TOL	0.202	-0.726	-0.060	0.764
SSI	-0.371	-0.684	-0.814	0.529

Table 8. Canonical loadings for the first and second canonicalvariables in the canonical discriminant analysis.

Table 9. Average of the groups on the first and second canonical variables in CDA.

	1 ^{**} Year		2 ⁴⁴ Year		
Group	First canonical variable	Second canonical variable	First canonical variable	Second canonical variable	
1	10.116	-4.308	4.497	2.194	
2	17.306	5.529	9.443	-2.656	
3	-32.480	0.933	-16.189	-0.635	

Drawing the scatter diagram using the first two canonical variables (Figs. 3-4) distinguishes three groups. Thereafter, the distances of the groups have been plotted by the scale of Mahalanobis distance (D^2) in Tables 10-11. All pairs of distances between groups were significant.



Fig. 3. The plot of first two canonical variables for genotypes classification (1st Year).



Fig. 4. The plot of first two canonical variables for genotypes classification (2nd Year).

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Table 10. Pairwise Squared Distances Between Groups (above diameter) and F* statistic (below diameter) in the canonical discriminant analysis (1st Year)

	Group 1	Group 2	Group 3	
Group 1	0	148.48	1842	
Group 2	16.19**	0	2500	
Group 3	200.94**	227.26**	0	

*Degrees of freedom of the numerator and denominator are 8 and 4, respectively.

 Table 11. Pairwise squared distances between groups (above diameter) and F* statistic (below diameter) in the canonical discriminant analysis (2nd Year).

	Group 1	Group 2	Group 3	
Group 1	0	47.99	435.97	
Group 2	5.23**	0	661.17	
Group 3	47.56**	60.10**	0	

*Degrees of freedom of the numerator and denominator are 8 and 4, respectively.

According to Figs. 3 - 4, in both years, the group 2 has been the best group. The genotypes of this group had the maximum values for the first canonical variable, where most of these genotypes had quantitative values for the second one, confirmed by the average of this group for both variables. Therefore, the genotypes of this group can be selected as suitable genotypes for stress and non-stress conditions (Table 12).

Table 12. Genotypes	in each of the gro	oups derived from the	ne canonical d	liscriminant analysis.

Group 2831 – Fajr – Sepidrood – SahelGroup 3Khazar- Hashemi – Alikazemi - Sangejo	Group 1	203 - 416 - 841 - Dorfak - Neda- SA13
Group 3 Khazar- Hashemi – Alikazemi - Sangejo	Group 2	831 – Fajr – Sepidrood – Sahel
	Group 3	Khazar- Hashemi – Alikazemi - Sangejo

Parallel coordinate plot (PCP) indicated the genotypes in each of the groups for all indices (Figs. 5-6). As a whole, the second group exhibited simultaneously larger tolerance indices along with lower sensitivity indices in both years.



Fig. 5. The parallel coordinate plot for three groups and all traits of interest (1st Year).

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Fig. 6. The parallel coordinate plot for three groups and all traits of interest (2nd Year).

In general, this method allowed better understanding the behavior of genotypes under stress. Notably, producing a high-yielding genotype under stress conditions was high due to the bearing or production capacities (or both) employing this method along with canonical variables. The first canonical variability was affected by high loads for tolerance and grain yield indices under stress and non-stress conditions (Yp and Ys). The canonical loads of sensitivity indices (SSI and TOL) were significant in the second canonical variable.

Therefore, high-yielding and tolerant genotypes should exhibit greater values in the first canonical variability, while lower in the second one. Such a distinction can be useful for breeding programs since it is possible to identify high yielding and tolerant genotypes or only tolerate genotypes for crossing with high-yielding genotypes. Using the canonical analysis for evaluation of drought stress tolerance in spring wheat, Safari *et al.* (2018) observed that the first canonical variables separate the genotypes based on yield potential and stress tolerance, while the second ones distinguish these genotypes from sensitive ones.

Correlation analysis

The results of the study in two succesive years indicated significant positive and high correlations between MP, GMP, HM, STI and grain yield under the stress and non-stress conditions (Tables 13-14). Therefore, The specified indices were considered as the best ones and indirect criteria for choosing tolerant genotypes.

Table 13. Contration coefficient among Drought tolerance indices and grain yield (1st Tear).										
YS	YP	MP	GMP	HM	TOL	SSI	STI			
YS	1									
YP	0.57 *	1								
MP	0.83 **	0.93 **	1							
GMP	0.91 **	0.85 **	0.99 **	1						
HM	0.96 **	0.76 **	0.95 **	0.99 **	1					
TOL	- 0.11 ns	0.75 **	0.46 ns	0.31 ns	0.16 ns	1				
SSI	-0.64 *	0.25 ns	-0.12 ns	-0.28 ns	-0.42 ns	0.81 **	1			
STI	0.91 **	0.84 **	0.98 **	0.99 **	0.99 **	0.28 ns	-0.30 ns	1		
ne * **: Not significant and significant at 0.05 and 0.01 probability layals, respectively										

Table 13. Correlation coefficient among Drought tolerance indices and grain yield (1st Year)

ns, *, **: Not significant and significant at 0.05 and 0.01 probability levels, respectively.

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YS YP MP GMP HM TOL SSI STI YS 1 YP 0.79** 1 0.94 ** 0.95 ** MP 1 GMP 0.97 ** 0.92 ** 0.99 ** 1 0.98 ** 0.99 ** 0.99 ** HM 0.89 ** 1 TOL - 0.26 ns 0.25 ns -0.01 ns -0.07 ns -0.11 ns 1 -0.77 ** SSI -0.90 ** -0.47 ns -0.72 ** -0.81** 0.54 *1 STI 0.65 * 0.58 * 0.64 * 0.65 * 0.65 * -0.55* 1 -0.13 ns

Table 14. Correlation coefficient among Drought tolerance indices and grain yield (2nd Year).

ns, *, **: Not significant and significant at 0.05 and 0.01 probability levels, respectively.

CONCLUSION

As a whole, this method made it possible to better understanding the behavior of genotypes under stress. Notably, the production of a high-yielding genotype under stress conditions is high due to the bearing capacity or production capacity (or both) using this method and the use of canonical variables. In this study, genotypes with high tolerance and high yield (high first canonical variable) and lower sensitivity (low second canonical variable) assigned in group 2 (831, Fajr, Sepidroud and Sahel) were found as superior genotypes in both stress and non-stress conditions.

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چکیدہ

تنش خشکی به عنوان یک عامل موثر کاهش تولید در گیاهان شناخته شده است که در این باره شناسایی و گسترش ژنوتیپهای سازگار به تنش یکی از راه کارهای غلبه بر شرایط نامساعد محیطی است. تجزیه تشخیص کانونیک (CDA) در ترکیب با روش تجزیه خوشهای برای بررسی پاسخ ۱۴ ژنوتیپ برنج با استفاده از شاخصهای تحمل به تنش، در دو شرایط آبیاری و قطع آبیاری در زمان گردهافشانی مورد استفاده قرار گرفت. نتایج CDA نشان دادند که اولین متغیر کانونیکی تحت تاثیر ضرایب زیاد برای شاخص تحمل به تنش (STI)، میانگین هارمونیک عملکرد (IMH)، میانیگن حسابی عملکرد (IM)، میانگین هندسی عملکرد (GMP)، عملکرد در شرایط نرمال (Yp) و عملکرد در شرایط تنش (Ys) بود. ضرایب کانونیکی شاخصهای حساسیت به تنش (SSI) و شاخص تحمل (IDT) در دومین متغیر کانونیکی ژنوتیپهای متحمل به تنش کانونیکی اول، ژنوتیپها را بر اساس پتانسیل عملکرد و تحمل به تنش و دومین متغیر کانونیکی ژنوتیپهای متحمل به تنش را از حساس متمایز کرد. نمودار پراکنش دو متغیر کانونیکی معنیدار، سه گروه را متمایز کرد و تمام جفت فواصل ماهالانوبیس میان گروهها معنیدار بودند. گروه دوم، به عنوان برترین گروه شناخته شد چرا که ژنوتیپهای این گروه از بیشترین مقدار برای متغیر کانونیکی اول برخوردار بودند و اکثر این ژنوتیپهای میلی کرد و مام جفت فواصل مودند. بنابراین ژنوتیپهای این گروه (۸۳۱، فجر، سپیدرود و ساحل) را میتوان به عنوان ژنوتیپهای مناس برای محیطهای واجد تنش و فاقد تنش انتخاب کرد.

*مولف مسئول

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