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Short Communication

Character association in mulberry under different magnitude of salinity stress

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Abstract: Information on interrelationships among traits is important for varietal development. Under stress conditions the magnitude of character association is known to change significantly. In mulberry such information on such aspects is lacking. Hence, the interrelationships of leaf yield and its component morphological, physiological and biochemical traits were investigated under four different salinity conditions using 11 genotypes. Correlation coefficient between leaf yield and its component traits was found changing significantly under different salinity stresses. Under normal condition leaf yield was found significantly and positively correlated with leaf size, root length, shoot length, protein content, and water use efficiency. However, under severe salinity imposed by 1.00% NaCl, significant correlation was observed with plant height, leaf size, shoot weight, root weight, root length, protein, nitrogen reductase (NRase) activity and water use efficiency (WUE) of the plant. Similarly, relationship of plant height with other traits also changed with increasing salinity. These changes in the interrelationships among the traits point to the need of different selection strategy for breeding varieties under normal and abiotic stress conditions.

Keywords: Mulberry, correlation coefficient, salinity, leaf yield.

الصفات المشتركة في ثمرة التوت تحت مقاييس وضغوط ملحية مختلفة

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ملخص: المعلومات المشتركة عن الصفات الوراثية تعتبر مهمة لاستنباط الأصناف الإنتاجية تحت ظروف النمو المجهد لتغير مقاييس الصفات المشتركة المعروفة بالتالي لتغيير صفاتها ايجابيا. في ثمرة التوت بعض المعلومات عن بعض الصفات تعتبر غير متوفرة وبالتالي فان المعلومات المشتركة لإنتاجية أوراق نبات التوت والصفات والمركبات الشكلية والفسيولوجية والصفات البيوكيميائية كانت محل الدراسة لحوالي أربع درجات تملح وإحدى عشر محصول ذو أنماط جينية مختلفة وجد أن معامل الارتباط من إنتاجية أوراق ثمرة التوت وصفاتها الوراثية المكونة له وجد أن هناك تغير كبير مع درجات التملح المختلفة وفى ظل حالة النمو الطبيعية لأوراق ثمرة التوت وجد أيضا ارتباط ايجابي مع مساحة الأوراق وطول القطاع الجزري واستطالة النمو الخضري والمحتوى البروتينى والكفاءة في استخدام المياه في عملية النمو ومع ذلك ومع درجات الملوحة العالية 1.00% NaCl وجد أن هناك علاقة أجابية مع استطالة النبات ومساحة الأوراق والوزن الخضري والوزن الجزئي واستطالة الجذور والبروتين واختزال عنصر (NRase) وكفاءة استخدام المياه في النبات وبالمثل هناك علاقة بين استطالة النبات مع الصفات الوراثية المختلفة قد تغيرت مع ارتفاع نسبة الملوحة وهذه التغيرات في العلاقات المشتركة بين الصفات الوراثية تشير إلى الحاجة الماسة لإستراتيجية انتخاب مختلفة لاختيار تربية الأصناف النباتية تحت الظروف الطبيعية وتحت ظروف النمو الغير حيوية.

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Introduction

Mulberry (*Morus*) of the family Moraceae is one of the most valued tree crops in several Asian countries like China, India, Korea, Bangladesh etc. due to its key role in sustaining the sericulture industry and thereby providing employments to a large number of people. Mulberry leaf is the only feed available for the silkworm *Bombyx mori*, hence, availability of good quality leaf has great impact on the sustainability and profitability of sericulture. Keeping this in view, breeding in mulberry mainly targets on developing varieties that grow well under divergent agro-climatic conditions to produce higher quantity of qualitatively superior leaf (Vijayan et al., 2008a, Vijayan, 2009). Leaf yield in mulberry is a complex trait contributed by many components such as plant height, number of branches, leaf retention capacity, nodal length and leaf weight (Banerjee et al., 2007; Sahu et al., 1995; Tikader and Kambli; 2008, 2009; Vijayan et al., 1997). Each of these component traits, in turn, is controlled by several genes, their interaction among themselves and with the environment. For instance, under drought conditions, the association between leaf yield and its component traits vary significantly (Susheelamma et al., 1998).

Naturally occurring salt affected soils cover more than a billion hectors (Flowers and Flowers, 2005) and out of the 275 million hectors of the agricultural lands 20% is affected by salinization (Ghassemi et al., 1995). The most economic and viable method of utilizing the salt affected land is by developing salt tolerant crops to cultivate there. Developing salt tolerant crop, however, is not easy as salt tolerance is a complex trait contributed by several associated traits. In order to develop salt tolerant varieties integration of knowledge on physiological, biochemical and genetic aspects of salt tolerance is essential (Ashraf and Foolad, 2007). High concentration of Na^+ in the soil inhibits the growth of most of the glycophytes, which include most of the crop plants including mulberry (Jaleel 2007, 2009). Thus, in order to distinguish the salt tolerant from the salt sensitive and to develop salt tolerant varieties, it is important to have adequate knowledge on the interrelationship of various characters in the plant under salt stress conditions because the selection indices varies under stress and non-stress conditions (Gholipouri et al., 2009). Therefore, information on the impact of salt stress on interrelationships of leaf yield and its component traits will be of much help to formulate appropriate breeding and selection strategies in mulberry. Since, India, China, and other sericulturally important countries have vast areas affected by salinity especially in the arid and semiarid regions, evolution of salt tolerant mulberry varieties are seen to be an essential part of the ongoing research. Although studies in other crops like barley (Kumar and Gupta, 1984), tef (Asfwa and Itanna, 2009) and wheat (El-Hendawy et al., 2009; Sinclair and Hoffmann, 2003) have shown that the magnitude of association among traits associated with plant growth and developments vary significantly under different growing conditions. Unfortunately, there is a fundamental gap in our understanding on the relationship between tolerance at low and high salinity levels in mulberry. This is an important problem while choosing the salinity level that will be used for screening the plants because the physiological mechanisms that prevent damage at low salt concentrations are not the same as those that contribute to tolerance at extremely high concentrations. Keeping this in view, the present investigation was undertaken with an objective to work out the interrelationships among different traits including morphological, biochemical and anatomical characters under different salinity levels.

Materials and Methods

Eleven mulberry genotypes comprising five parents such as English black, Rotundiloba, Mandalaya, Tollygunj, and C776 along with six hybrids developed from these parental genotypes were cultured under five different salinity levels viz., EC 1.6, 6.5, 10.1, 14.1 and 19.2 dSm^{-1} respectively, developed via addition of 0.0%, 0.25%, 0.50%, 0.75% and 1.00% NaCl as described earlier (Vijayan et al., 2008a). Morphological traits such as number of branches, shoot height, leaf size, leaf fresh weight, leaf dry weight, moisture (%), number of nodes per branches, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and root-shoot ratio were recorded by planting three months old saplings on earthen pots as described earlier (Vijayan et al., 2008a,b). Biochemical assays for protein, soluble sugar, proline, phenol, nitrogen reductase (NRase), chlorophyll-a, chlorophyll-b, carotenoids, total chlorophyll, K^+ : Na^+ ratio, and Na^+ and K^+ contents were conducted as described earlier (Vijayan et al., 2008a). The photosynthesis related characters such as photosynthetic rate, internal CO_2 concentration, transpiration rate and physiological water use efficiency (WUE) were studied on 60th day using LICOR 6200 portable photosynthetic meter (LI-COR Biosciences GmbH, Siemensstrasse 25A, D-61352 Bad Homburg, Germany). Fifth leaves from the apical portion of twigs were used for measurements as reported earlier (Das et al., 1999). Statistical analyses were carried out in SPSS/PC+ 10.0 program (M.J. Norusis, SPSS Inc., Chicago).

Results and Discussion

The association of different characters with the principle trait i.e., leaf as assessed by their correlation coefficients clearly suggest that the intensity of the association among these traits changes with the severity of the salt stress (Table 1). Under normal cultural conditions (control set in this case), the leaf yield was seen positively and significantly correlated with leaf size, root length, shoot length, and shoot fresh and dry weights. Although, the number of branches showed positive correlation but it was not significant which was in contradiction to some of the earlier observations that number of braches in mulberry is significantly correlated with leaf yield (Sahu et al., 1995; Vijayan et al., 1997). This could be due to the immaturity of the plant which was used for current observations as in general mulberry needs at least three to four years for better establishment and proper expression of characters. Recently, Tikader and Kamble (2008) observed almost similar results in an experiment with 25 germplasm accessions. They also found a positive but non-significant correlation between the number of branches and the leaf yield. However, leaf size and shoot lengths were highly correlated with leaf yield that agreed with most of the earlier observations (Tikader and Kamble; 2008, 2009; Vijayan et al., 1997). Under mild salinity imposed by 0.25% of NaCl, number of branches, leaf size, root length, shoot length and shoot weight were highly positively correlated with leaf yield. But under severe salt stress imposed by 1.00% NaCl, leaf size, leaf weight, root length, shoot length, root weight and shoot weight were positively and significantly associated with leaf yield. This clearly points to the fact that while selecting for salt tolerance, emphasis need to be give to root and shoot lengths. Some traits like moisture content showed negative correlations but none of them was found significant. The plant height also showed significant changes in its relationship with other component characters. Under normal condition only a few traits such as leaf dry weight, and root dry weight showed significant correlation with shoot height. But under high salinity almost all the morphological traits studied showed significant correlation (Table 2). In an earlier study under drought stress, Susheelamma et al. (1998) also found similar type of changes in the correlation of leaf yield with the plant height. However, this is the first attempt in mulberry to investigate the correlation between leaf yield and its component characters under salt stress conditions. Studies in other crops also revealed such changes under severe salinity (El-Hendawy et al., 2009; Kumar and Gupta, 1984). For example, correlation and path coefficient studies on 15 varieties of barley on saline and normal conditions revealed that yield was strongly correlated with number of tillers, 100 grain weight on normal conditions and germination and number of tillers in saline (Kumar and Gupta, 1984). Similarly, Gholipouri et al. (2009) and Sanjeri et al. (2006) also found significant changes in the character association in wheat under stress and non-stress conditions.

Table 1. Correlation coefficient of the principal trait "leaf yield" with its component traits of morphological, biochemical and photosynthetic characters in mulberry under different salinity levels.

Characters	**NaCl (%)**				
	0.00	**0.25**	**0.50**	**0.75**	**1.00**
No. of branches	0.236	0.483**	0.278	0.236	0.189
Leaf size	0.475**	0.566**	0.336	0.414*	0.390*
Leaf fresh Weight	-0.007	0.227	0.359*	0.449**	0.674**
Leaf dry weight	-0.028	0.237	0.463**	0.485**	0.696**
Moisture (%)	0.004	-0.083	-0.244	0.010	-0.211
No. of nodes	-0.138	-0.047	-0.113	0.086	0.370*
Root length	0.480**	0.414*	0.576**	0.570**	0.589**
Shoot length	0.453**	0.617**	0.608**	0.329	0.517**
Shoot fresh weight	0.822**	0.808**	0.694**	0.638**	0.739**
Shoot dry weight	0.734**	0.804**	0.692**	0.570**	0.761**
Root fresh weight	0.245	0.252	0.531**	0531**	0.594**
Root dry weight	0.245	0.316	0.622**	0.601**	0.659**
Root-shoot ratio	-0.257	-0.302	-0.046	0.064	0.178
Protein	0.441**	0.333	0.396*	0.301	0.381*
Sugar	-0.123	0.077	0.227	0.353*	0.147
Proline	-0.289	0.182	-0.150	-0.102	0.198
Phenol	-0.303	-0.285	-0.368*	0.100	0.034
NRase	0.162	0.203	0.364*	0.536**	0.124
Chlorophyll-a	-0.094	0.280	0.126	0.074	0.373*
Chlorophyll-b	-0.079	0.263	-0.178	0.001	0.192
Carotenoids	-0.283	0.271	0.176	0.236	0.564**
Total chlorophyll	-0.123	0.319	0.045	0.057	0.326
K^+ : Na^+ ratio	-0.081	0.255	0.173	0.272	0.486**
Na^+	0.010	-0.208	-0.141	-0.332	-0.345*
K^+	-0.031	0.108	0.064	0.019	0.373*
Photosynthetic rate	0.366*	0.551**	0.463**	0.434*	0.503**
Internal CO_2	-0.235	-0.217	-0.257	-0.407*	-0.191
Stomatal Cond.	-0.066	-0.123	-0.227	-0.143	0.038
Transpiration	-0.314	0.074	-0.011	0.124	0.032
WEU	0.318	0.552**	0.493**	0.423*	0.028
	n=33	n=33	n=33	n=33	n=33

Significance *p<0.05, **p<0.01

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Table 2. Correlation coefficient of shoot height with other morphological , biochemical and photosynthetic characters in mulberry under different salinity levels.

Characters	NaCl (%)				
	0.00	**0.25**	**0.50**	**0.75**	**1.00**
No. of branches	0.202	0.074	0.012	0.400*	0.251
Leaf size	0.018	0.182	0.088	0.288	0.314
Leaf fresh Weight	-0.330	0.213	0.151	0.086	0.319
Leaf dry weight	-0.344*	0.191	0.175	0.027	0.355*
Moisture (%)	0.287	0.025	-0.017	0.273	-0.254
No. of nodes	0.343*	0.088	0.007	0.513**	0.556**
Root length	0.067	0.458**	0.712**	0.696**	0.389*
Shoot fresh weight	0.409*	0.585**	0.671**	0.414*	0.638**
Shoot dry weight	0.221	0.376*	0.707**	0.403*	0.594**
Root fresh weight	0.086	0.458**	0.744**	0.492**	0.574**
Root dry weight	0.453**	0.617**	0.608**	0.329*	0.517**
Root-shoot ratio	-0.245	-0.022	0.063	0.071	-0.105
Proteins	0.035	0.085	0.294	0.516**	0.541**
Sugar	0.029	-0.067	0.087	0.221	0.461**
Proline	-0.148	-0.068	-0.183	-0.059	-0.141
Phenol	-0.077	0.123	-0.184	0.138	0.462**
NRase	-0.115	0.099	0.302	0.441**	0.475**
Chlorophyll-a	0.255	0.228	0.145	0.436*	0.455**
Chlorophyll-b	0.082	0.231	0.039	0.449**	0.430*
Carotenoids	0.145	0.379*	0.196	0.440**	0.559**
Total chlorophyll	0.119	0.264	0.103	0.502**	0.481**
K^+ : Na^+ ratio	-0.190	0.134	0.258	0.447**	0.459**
Na^+	0.103	-0.030	0.116	-0.528**	-0.522**
K^+	0.094	0.197	0.168	0.060	0.243
Photosynthetic rate	0.029	0.491**	0.194	0.431*	0.383*
Internal CO_2	-0.208	-0.157	0.053	-0.233	-0.316
Stomatal Cond.	-0.269	-0.130	-0.262	0.090	0.239
Transpiration	-0.233	0.213	0.352*	0.132	0.225
WEU	0.035	0.228	0.218	0.244	0.222
	n=33	n=33	n=33	n=33	n=33

Significance * P<0.05, ** p<0.01

Regarding the correlation of biochemical parameters with leaf yield, under normal salt level only proteins showed significantly positive correlation with leaf while other traits showed negative correlations with leaf yield (Table 1). However, under salt stress conditions, proteins, chlorophyll-a, total chlorophyll contents in the leaf showed significantly positive correlations. Proline, NRase and Na^+ showed significantly negative correlations. The photosynthetic rate was significantly and positively correlated with leaf yield in all the cultural conditions. Under high salt stress, water use efficiency and transpiration rates showed significantly positive correlation with leaf yield. Thus, it is clear that under slat stress condition the character association in mulberry differed significantly from that under normal conditions. Susheelamma et al. (1998) also found similar type of changes in the correlation of leaf under drought stress.

Biochemical characters also showed significant correlation with leaf yield (Table 1). Under normal condition the leaf was positively significantly correlated with soluble proteins. Soluble sugar, on the other hand, showed negative correlation in the normal condition but showed positive correlation under higher saline conditions. Proline did not show any significance with the leaf yield except under 1.00% NaCl where it exhibited a negative correlation. NRase showed positive correlation with leaf yield. Though chlorophyll-a was positively correlated with leaf yield, the other two pigments did not show much correlation with the leaf yield. Concentrations of sodium showed negative correlation with the leaf yield. The detrimental effects of high concentration of Na^+ in the soil on growth, leaf morphology and biochemical components have been well documented (Koyro, 2000). The toxicity of Na^+ in plants is caused by osmotic stress, enzyme inhibition and competition with K^+ (Tester and Davenport, 2003).

Photosynthetic rate showed a very strong positive correlation with the leaf yield. The other three parameters i.e., internal CO_2 concentration, stomatal conductance and transpiration rate did not show any significant correlation with the leaf yield except that of water use efficiency, which had a very strong positive correlation with the leaf yield. Similar type of strong correlation between WUE and yield was observed in barley (Febrero et al., 1994). Likewise, photosynthetic rate was positively correlated with the shoot dry weight of cotton (Brugnoli and Lauteri, 1990). A reduced photosynthetic rate is the general observation in plants, which could be due to the result of stomatal closure or more directly through the toxic effect of salinity on the photosynthetic apparatus (Praxedes et al., 2009). In an earlier experiment, Lakshmi et al. (1996) reported significant reduction in photosynthesis due to reduced stomatal conductance (g_s) in mulberry under salinity, this reports completely agrees with the findings in the present study.

Thus, from the present study it can be concluded that interrelationship among the important leaf yield contributing traits changes under different stress conditions. This necessitates changes in the selection criteria for mulberry breeding under low and high salt concentrations.

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