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Genetic control of aluminium tolerance in *okra* (*Abelmoschus esculentus* (L.) Moench)

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ABSTRACT

Okra (*Abelmoschus esculentus*) genotypes 'Parbhani Kranti' and 'Arka Anamika' resistant to Al^{3+} were crossed to two aluminium-sensitive genotypes, 'CO-203' and 'Punjab-7' to determine the nature of inheritance of resistance. The parents, F_1, F_2 and F_3 generations were grown in nutrient solution containing 10 mg/l Al^{3+} for hematoxylin staining of root tips and classified for tolerance. The segregation ratios between the resistant and sensitive genotypes in the F_2 ($n = 1071$) and F_3 ($n = 335$) were 15: 1 and 7: 8:1, respectively. These results indicated that Al^{3+} resistance is controlled by two dominant genes. This is the first report of Al^{3+} resistance in okra.

1. Introduction

Okra (*Abelmoschus esculentus* (L.) Moench is one of the important vegetables of the tropical and subtropical areas. Almost all parts of okra plant are consumed, e.g. fresh okra fruits are used as vegetable, roots and stems are used for clearing the cane juice (Chauhan, 1972), and leaves and stems are used for making fibres and ropes (Jideani and Adetula, 1993). In India, it is cultivated in 0.45 million hectares area with the production of 4.8 million tones. The major okra producing states in India are West Bengal, Bihar, Orissa, Andhra Pradesh, Gujarat, Jharkhand, Chhattisgarh, Maharashtra, Assam and Uttar Pradesh (NHB, 2010).

In India, 49 million hectares of land is affected by soil acidity of which 24 million hectares have pH below 5.5 (Mandal, 1997). Aluminium toxicity (Al^{3+}) is a serious problem limiting crop productivity in the low pH acidic soils (pH < 5.5) that are difficult to lime (Singh et al., 2011b). Aluminium becomes soluble at low pH (<5.5), inhibiting root growth and severely reducing yield (Krill et al., 2010). Some plant species have developed different mechanisms to minimize the harmful effects of Al toxicity. The most documented mechanisms of Al resistance are the secretion of anions of organic acids from the roots (Ryan et al., 2001; Kochian et al., 2005).

It is possible to detoxify Al in surface soil by liming to pH 5.5 or above. However, liming is not a remedy for subsoil acidity and it is not always economically feasible (Tesfaye et al., 2001). Therefore, the most appropriate strategy to overcome Al toxicity is to select or breed genotypes with tolerance to aluminium toxicity (Singh and Raje, 2011). For this, sources of tolerance and pattern of inheritance need to be identified. In order to investigate the genetics of resistance it is necessary to screen and measure the resistance of aluminium toxicity in large number of genotypes by using rapid, effective and reproducible screening techniques. The most common technique is hematoxylin staining (Polle et al., 1978; Camargo, 1988; Luo and Dvorak, 1996; Tang et al., 2000; Singh et al., 2009; Singh and Chaudhary, 2010; Singh and Raje, 2011). This technique makes it possible to detect the levels of resistance visually in a large number of genotypes without destroying the root apical meristems.

Genetic variation for Al^{3+} resistance exists within okra germplasm (Singh and Sureja, 2008). Several studies have demonstrated aluminium (Al) tolerance to be a complex (Lafever and Campbell, 1978; Aniol and Gustafson, 1984; Aniol, 1990; Arajo et al., 1992) as well as simple (Rhue et al., 1978; Singh and Chaudhary, 2010; Singh and Raje, 2011) trait. However, the nature of inheritance of Al^{3+} resistance is not yet fully understood. It is important to determine the genetics of the Al^{3+} resistance and explore the possibility of utilizing the trait in future breeding programmes.

2. Materials and methods

2.1. Plant materials

The parental lines such as 'Parbhani Kranti', 'Arka Anamika', 'CO-203' and 'Punjab-7' were selected on the basis of previous studies on the assessment of aluminium tolerance (Singh and Sureja, 2008). Seedlings were tested in the completely randomized design with

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two replications for evaluation of parental lines. The four parental strains were crossed in all possible combinations (including reciprocals). The crosses were made by hand as described by Joshi et al. (2002). The F_2 seeds of the cross were produced in pots by bagging the F_1 plants in butter paper bags. The F_3 seeds were then harvested from each F_2 plant individually.

2.2. Evaluation of aluminium tolerance

The parental genotypes were confirmed for aluminium sensitivity by hematoxylin staining and root re-growth in nutrient solution. The score of hematoxylin staining was compared to the score obtained from root re-growth measures. Subsequently, the aluminium response of parental genotypes, and plants in the F_1, F_2, and F_3 generations was assayed only by hematoxylin staining in nutrient solution under controlled environment [32–33 °C, 12/12 h (light/dark), and 80% relative humidity]. The hematoxylin staining (Polle et al., 1978; Singh et al., 2009) has been routinely used in genetic studies (Minella and Sorrells, 2002; Singh and Chaudhary, 2010; Singh and Raje, 2011). The procedure as given by Polle et al. (1978) with partial modifications was used in the present study to screen okra seedlings for aluminium tolerance. Seeds were surface sterilized with 0.1% $HgCl_2$ for 2–3 min and rinsed thoroughly with distilled water and then transferred to filter paper in the growth chamber for germination. After 1 week the seedlings were transferred to plastic containers in nutrient solution (4.0 mM $CaCl_2$, 6.5 mM KNO_3, 2.5 mM $MgCl_2$, 0.1 mM $(NH4)_2SO_4$, 0.4 mM NH_4NO_3) that was adjusted to pH 4.5 with 0.1 M HCl or 0.1 M NaOH solutions. Seedlings were kept in the above nutrient solution for 2 days under continuous light and aeration. Thereafter, the seedlings were maintained for 24 h on the fresh nutrient solution containing 10 mg/l Al concentration, because this concentration gave best discrimination for resistance and sensitivity in okra. The root of seedlings were then placed in aerated distilled water and washed for 30 min to remove excessive aluminium on the root surface. The roots along with seedlings were then immersed in hematoxylin solution of 2 g/l and 0.02 g/l KIO_3 for 15–30 min. The roots were washed again for 30 min in deionized water three times to remove excess of stain. Each seedling was visually scored for the intensity of staining of the primary root tips. Seedlings of the parent genotypes, F_1, F_2 and F_3 generations were visually classified on the basis of degree of staining of the root tips and were graded as complete (3) and partial staining (1), or non-stained (0). The non-stained and partially stained seedlings were grouped together as tolerant plant, and those deeply stained as sensitive. This was done because the resistant and sensitive parents showed absence or partial, and complete staining. The segregation ratios of the resistant and sensitive plants in the F_2 generation and F_3 progenies were tested by χ^2 analysis.

3. Results and discussion

The hematoxylin staining score revealed a significant negative correlation ($r = -0.88^{**}$ indicates that correlation between staining score and root re-growth is highly significant.) with root re-growth/regeneration (Fig. 1). This confirms that deeper hematoxylin reflects high degree of Al^{3+} sensitivity and lower level or absence of staining is indicative of higher aluminium resistance. Therefore, either of the two screening procedures can be used for evaluation of genotypes as well as segregating populations for Al^{3+} resistance. However, the hematoxylin method is simple and requires minimal space, cost, labour as compared with the root re-growth/regeneration method. The data in the present studies revealed that the parents 'Parbhani Kranti' and 'Arka Anamika' showed no staining or partial staining with a mean stain score of

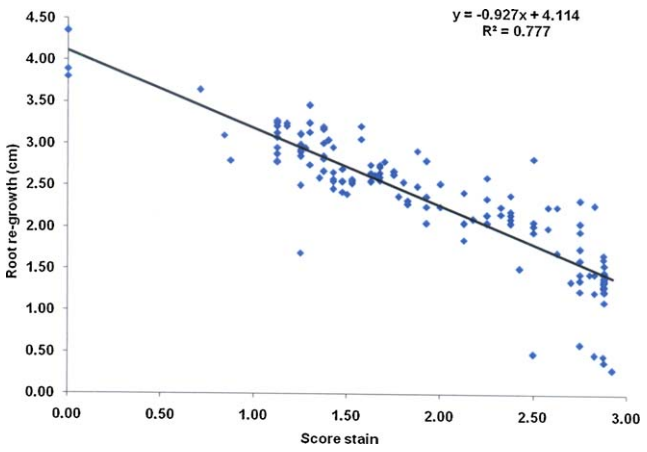

Fig. 1. Correlation between the score of hematoxylin staining and root re-growth in the nutrient soltion containing various concentration of Al (5, 10, 15 and 20 mg/l aluminium).

Table 1
Genotypes used in the study, their stain mean scores and root re-growth.

Genotype	Mean stain score	Al-reaction
Parbhani Kranti	0.59	Tolerant
Arka Anamika	0.75	Tolerant
CO-203	3.00	Sensitive
Punjab-7	3.00	Sensitive

0.59 (SE 0.085) and 0.75 (SE 0.080), respectively, thus showing a high degree of tolerance to aluminium toxicity compared to the sensitive genotypes, viz., 'CO-203' and 'Punjab-7' which showed intense staining with a mean stain score of 3.0 (SE 0.00) under controlled nutrient solution study (Table 1 and Fig. 2).

The F_1 seedlings from the crosses 'Parbhani Kranti'/'CO-203', 'CO-203'/'Parbhani Kranti', 'Parbhani Kranti'/'Punjab-7', 'Punjab-7'/'Parbhani Kranti', 'Arka Anamika'/'CO-203', 'CO-203'/'Arka Anamika', 'Arka Anamika'/'Punjab-7', 'Punjab-7'/'Arka Anamika', 'Parbhani Kranti'/'Arka Anamika' and 'Arka Anamika'/'Parbhani Kranti' exhibited resistance reaction with absence or partial staining of roots. The F_1 progenies of crosses 'CO-203'/'Punjab-7' and 'Punjab-7'/'CO-203' resulted in sensitive reaction (Table 2). The F_1 progenies of crosses between resistant and sensitive parents always resulted in resistant reaction as staining in such hybrids was similar to the resistant parent. This indicates that aluminium tolerance is dominant over sensitive reaction. The F_1 progeny of the cross 'Parbhani Kranti'/'Arka Anamika' (tolerant × tolerant) and 'CO-203'/'Punjab-7' (sensitive × sensitive) were all resistant and

Table 2
Reaction to aluminium toxicity of F_1 progenies of crosses in both directions (direct and reciprocal) of okra genotypes based on hematoxylin staining.

Cross	F_1 reaction	Phenotypic frequencies		Total
		Tolerant	Sensitive	
Parbhani Kranti/CO-203	T	64	0	64
CO-203/Parbhani Kranti	T	58	0	58
Parbhani Kranti/Punjab-7	T	50	0	50
Punjab-7/Parbhani Kranti	T	65	0	65
Arka Anamika/CO-203	T	60	0	60
CO-203/Arka Anamika	T	64	0	64
Arka Anamika/Punjab-7	T	70	0	70
Punjab-7/Arka Anamika	T	65	0	65
Parbhani Kranti/Arka Anamika	T	65	0	65
Arka Anamika/Parbhani Kranti	T	60	0	60
CO-203/Punjab-7	S	0	72	72
Punjab-7/CO-203	S	0	60	60