Genotypic variation in auxin-induced rooting response of detached tomato leaves: an innovative approach for indirect evaluation of yielding ability in tomato (*Solanum lycopersicum* L.)

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In this study, the extent of genetic variation in auxin-induced rooting response of detached tomato leaves has been assessed and correlated with yielding ability. Fully expanded, healthy and disease-free compound leaves were excised and treated with 500 ppm aqueous indole butyric acid solution for 45 sec, then washed thoroughly with tap water and kept in beakers filled with distilled water. The leaves were examined randomly for the number of roots induced, length of the longest root, fresh root weight and frequency of rooted leaves to determine variation in auxin response of the genotypes. Auxin-treated detached leaves of 22 tomato genotypes showed wide variation in their root traits. The genotypic coefficient of variation for the number of roots, root length, fresh root weight and frequency of rooted leaf cuttings was 45.43, 11.72, 38.45 and 31.66 respectively. Auxin sensitivity index (ASI) of the genotypes ranged from 0.0 to 3.0. A significant positive correlation (0.647) was observed between ASI and fruit yield. This study reveals that genetic variation in the auxin-induced rooting response of detached tomato leaves could be used as an indicator for screening high-yielding tomato genotypes. This innovative approach is simple, rapid and inexpensive.

Keywords: Auxin, detached leaves, rooting, screening, tomato, yield.

LEAVES are important plant organs in which photosynthesis is carried out to provide food for the sustenance of life. Maintenance of leaves in living conditions for various periods after detachment from the mother plant is known as detached leaf culture[1]. Leaves without axillary buds detached from the mother plant can survive for varying lengths of time after treatment with auxin under simple cultural conditions (even in the absence of complex media and without any sterilization as required for *in vitro* tissue/organ culture). Genetic variability in the base population is a pre-requisite for selecting superior genotypes over the existing ones. Since auxins as plant hormones are known to control growth and development, it is logical to expect that differences in auxin balance may form an important part of the physiological mechanism through which the genotypes exert their action for genetic variation. In the present study, indole butyric acid (IBA) of the auxin group that is known to induce roots in stems and leaf cuttings was selected. The use of IBA in inducing roots in cuttings of different vegetables has been reported by several researchers[2–4].

Detached leaf culture in tomato was used to screen disease-resistant genotypes against late blight[5], early blight[6] and phytophthora[7]. Detached leaf culture in rice was also used to assess the stay green trait[8]. High yield is the most important of the plant breeding objectives. Newly developed crop genotypes by the breeder(s) need field trials such as IET (initial evaluation trial), AVT-I and AVT-II (advanced varietal trials) for the selection of high-yielding genotypes. These trials involve considerable effort and expenditure like land, labour, money, manpower, etc. along with time. However, these trials are a necessity and cannot be avoided. It would be of great help and immense value if some method(s) could be developed to screen genotypes for high yield before taking them to multi-location trials. In the present study, detached leaf culture has been used as an indirect approach for preliminary evaluation of the yielding ability in tomato genotypes.

To the best of our knowledge, there are no studies on the use of IBA in inducing roots in detached tomato leaves to evaluate the yielding ability in tomato genotypes indirectly. Therefore, the present study was undertaken to assess variation in auxin response of tomato genotypes by inducing roots in detached leaves and find its relationship with yielding ability.

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Table 1. Estimates of genetic variability parameters for auxin-induced root traits of detached tomato leaves (mean of two years' data)

Genetic parameters	Number of roots per leaf	Length of the longest root (cm)	Fresh root weight (mg)	Frequency of rooted leaves
Mean sum of square	273.36**	1.342**	4746.36**	0.158**
Mean	12.8	6.06	55.0	0.67
Range	2–40	3–12.5	6–190	0.20–1
σ^2g (genotypic variance)	33.82	0.505	447.30	0.045
σ^2p (phenotypic variance)	49.43	0.595	603.64	0.067
GCV (genotypic coefficient of variation)	45.43	11.72	38.45	31.66
PCV (phenotypic coefficient of variation)	54.92	12.73	44.67	38.63
Heritability (%)	68.41	84.87	74.10	67.16
Genetic advance (GA) as percentage of mean	76.94	22.29	68.09	53.32

**Significant at 1% probability level.

Materials and method

Experimental materials

Twenty-two determinate tomato genotypes were chosen for this study. Tomato seeds were collected from the All India Coordinated Research Project (AICRP) on Vegetable Crops, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar, Odisha, India.

Experiment in detail

The field experiment was conducted during 2017–18 and 2018–19 at AICRP on vegetable crops, OUAT, Bhubaneswar following a completely randomized design (CRD) with three replications. Seeds of all the genotypes were sown in raised nursery beds during the first week of November. Twenty-five-day-old seedlings were transplanted into a poly pot filled with pot mixture. All cultural practices were uniformly followed to raise the crop successfully. Fruit yield/plant was recorded at the ripening stage. For assessing auxin response of genotypes, fully expanded, healthy and disease-free compound leaves were excised from the fifth to the eighth node 40 days after transplanting and were treated with 20 ml of 500 ppm aqueous IBA solution for 45 sec, then washed thoroughly with tap water and kept in beakers filled with distilled water. Five leaves were examined randomly on the tenth day of treatment for the number of roots induced, length of the longest root (cm), fresh root weight (g) and frequency of detached leaves producing roots. The laboratory experiment was conducted at the Department of Plant Breeding and Genetics, Odisha University of Agriculture and Technology, Bhubaneswar during 2017–18 and 2018–19, following CRD with three replications. The mean data were subjected to different statistical analyses.

Statistical analysis

The data collected for each quantitative trait were subjected to analysis of variance (ANOVA) using SAS version 9.3, after testing the ANOVA assumptions. Before pooling the data across environments, a test of heterogeneity for error of variance was done. The difference between treatment means was compared using CD value at a 1% probability level. The phenotypic and genotypic variances and coefficients of variation were estimated from ANOVA according to the method suggested by Singh and Chaudhary[9], heritability in a broad sense (H) by Burton and de Vane[10] and genetic advance (GA), i.e. the expected genetic gain was calculated using the procedure given by Johnson *et al.*[11].

Estimation of auxin sensitivity index

Auxin response of tomato genotypes was expressed in terms of auxin sensitivity index (ASI), which was calculated as follows. The root traits under study were suitably coded as '0' and '1' for below average and above average values respectively. Then the coded values of all the characters were added to obtain ASI[12].

Analysis of relationship between ASI parameter and yield

The relationship between auxin response and yielding ability was established following Pearson's method of correlation, as described by Panse and Sukhatme[13].

Results and discussion

Estimation of genetic parameters

The extent of genetic variability in root traits of detached tomato leaves was confirmed from the estimates of genetic parameters. ANOVA indicated significant variation among the tomato genotypes with respect to the number of roots per leaf, length of the longest root, fresh root weight and frequency of rooted leaf cuttings (Table 1), suggesting that the genotypes are genetically divergent from each other.

Table 2. Auxin-induced root traits of detached tomato leaves of high- and low-yielding genotypes (mean of two years' data)

Serial no.	Genotype	Fruit yield/ plant (g)	Auxin-induced root traits: No. of roots per leaf cutting	Length of the longest root (cm)	Fresh root weight (g)	Frequency of rooted leaf cutting	Auxin sensitivity index (ASI)
	Above average yielder						
2	V2. BT 10	1611.50	6.0	5.50	34.0	0.90	1.0
4	V4. BT-101	2164.00	13.0	7.10	139.0	1.00	2.5
6	V6. BT-136	1742.00	21.0	6.60	190.0	0.90	2.0
7	V7. BT-317	1881.00	30.0	5.00	80.0	1.00	2.5
8	V8. BT 12-2	1776.50	18.0	6.40	64.0	0.80	2.0
10	V10. BT 428-3	1950.50	4.5	4.00	16.0	1.00	1.0
12	V12. BT 506-1	1661.00	22.0	7.50	62.0	1.00	3.0
14	V14. BT 17-2-5	1927.50	10.5	6.30	9.0	0.30	0.5
15	V15. BT 19-1-1-1	1879.50	40.0	9.50	180.0	0.50	2.0
20	V20. Arka Vikas	1565.50	11.0	6.30	94.0	0.70	1.5
22	V22. Arka Rakshak	2437.00	19.2	6.84	77.0	0.80	2.0
	Mean	1871.65	17.74	6.45	85.91	0.81	1.81
	Below average yielder						
1	V1. BT 1	970.50	4.0	6.60	18.0	0.40	0.5
3	V3. BT-17	1282.50	2.0	3.00	6.0	0.50	0.0
5	V5. BT-106	1119.50	6.5	5.50	24.0	0.60	0.0
9	V9. BT 112-1	1385.00	5.0	7.00	11.0	0.80	1.5
11	V11. BT 442-2	1427.50	5.0	12.50	40.0	0.70	1.5
13	V13. BT 12-3-2	974.50	10.2	4.60	7.0	0.60	0.0
16	V16. BT 22-4-1	1098.00	12.3	5.70	20.0	0.70	0.5
17	V17. BT 306-1-2	1457.00	11.9	5.40	14.0	0.60	0.0
18	V18. BT 429-2-2	1178.50	14.0	4.60	82.0	0.40	2.0
19	V19. BT 433-2-3	1216.50	9.3	4.10	21.0	0.20	0.0
21	V21. Pusa Ruby	718.50	5.6	3.40	12.0	0.40	0.0
	Mean	1166.17	7.8	5.67	23.18	0.53	0.55
	Grand mean	1519.32	12.8	6.06	55.00	0.67	1.18

As mentioned in Table 1, the phenotypic coefficient of variation (PCV) was relatively more than the genotypic coefficient of variation (GCV) for all traits, indicating the influence of environment in the expression of different root characters. The highest GCV (45.43) and PCV (54.92) were observed in the number of roots per leaf, indicating that more variability is present in the population for this character (Table 1), followed by fresh root weight (GCV – 38.45; PCV – 44.67). The highest heritability was observed for the length of the longest root (84.87) and the lowest for the frequency of rooted leaves (67.16). The highest GA as a percentage of the mean was recorded in the number of roots per leaf (76.94). For root length, heritability was high (84.87) but GA as percentage of mean was low (22.29), which indicated that the trait is governed by non-additive genes. In case of number of roots per leaf, moderate heritability (68.41) was accompanied by high GA as percentage of mean (76.94), and this revealed that the character is governed by additive gene effects.

Variation in auxin-induced root traits

Roots that appeared in detached leaves of 22 tomato genotypes varied in their number, length, fresh weight and frequency (Table 2). The number of roots per leaf varied from 2.0 to 40.0, with an average of 12.8 (Table 2). Seven genotypes produced above-average (12.8) number of roots and rest of the genotypes had below-average number of roots. The length of the longest root ranged from 3.0 to 12.5 cm, with an average of 6.06 cm. The longest root was observed in genotype BT 442-2 (12.50 cm) and it was significantly greater than all other genotypes. The shortest root length was observed in BT 17 (3.0 cm). The fresh root weight of the genotypes varied from 6.0 to 190.0 mg. Genotype BT 136 recorded the maximum fresh root weight (190 mg), followed by BT 19-1-1-1 (180 mg). BT 17 recorded a minimum fresh root weight (6.0 mg). Frequency of rooted leaves ranged from 0.20 to 1.00 with an average of 0.67. The maximum frequency of rooted leaves was observed in BT 101 (1.00), BT 317 (1.00), BT 428-3 (1.00) and BT 506-1 (1.00). Auxin response of the genotypes was expressed in terms of ASI. The ASI value of the genotypes ranged from 0 to 3.0, with an average of 1.18. Some genotypes having above-average yield showed anomalous behaviour in their auxin-induced rooting response, which may be due to genotype and environment interaction.

Analysing the relationship

Fruit yield/plant of 22 tomato genotypes varied from 718.50 to 2437.00 g with an average of 1519.32 g (Table 2). Arka Rakshak recorded the highest yield of 2437.00 g, which was at par with BT 101 (2164.00 g). Pusa Ruby recorded the lowest yield (718.50 g), followed by BT 1 (970.50). Genotypes having above-average yield (>1519.32 g) were considered as high or above-average yielders (AAYs). Eleven out of 22 genotypes had above-average yields (V2, V4, V6, V7, V8, V10, V12, V14, V15, V20 and V22) and the rest were low yielders. The mean number of roots per leaf, length of the longest root, fresh root weight and frequency of rooted leaf cuttings for the high-yielding group were 17.74, 6.45 cm, 85.91 mg and 0.81 respectively, whereas in the case of a low-yielding group it was 7.80, 5.67 cm, 23.18 mg and 0.53 respectively (Table 2). ASI value of high- and low-yielding groups was 1.81 and 0.55 (Table 2). These results revealed that most of the high-yielding genotypes had high auxin response compared to low-yielding genotypes.

Tomato genotypes were classified into two classes based on their ASI value (Table 2). The genotypes having above-average ASI values (>1.18) were included in the auxin-sensitive group (or high auxin response group, HAR) and those having below-average ASI values (<1.18) were included in the auxin-tolerant group (or low auxin response group, LAR).

Eleven genotypes, namely V4, V6, V7, V8, V9, V11, V12, V15, V18, V20 and V22, were included in the HAR class and the rest 11 genotypes in the LAR class. Eight out of 11 genotypes of the HAR class were AAYs (frequency = 0.73) and three out of 11 genotypes present in the LAR class were AAYs (frequency = 0.27). Conversely, the response of high yielders to auxin indicated that eight out of 11 AAYs had high ASI value (frequency = 0.73) and three out of 11 AAYs had low ASI value (frequency = 0.27). This result indicated that the auxin response of detached leaves of genotypes could be used for indirect evaluation of yielding ability in tomatoes.

The relationship between yielding ability and auxin response was analysed by a correlation study. Correlation analysis showed that the ASI value had a significant positive correlation (0.647) with fruit yield. Correlation of individual root traits was done in order to determine which single root parameter could provide some indication for preliminary screening of high-yielding tomato genotypes. It was observed that the number of roots per leaf, fresh root weight per leaf and frequency of rooted leaf cuttings exhibited a significant and positive correlation of 0.490, 0.516 and 0.551 with yield respectively, whereas length of the longest root showed only positive correlation of 0.319. This result revealed that all the root traits had a positive correlation with yield. The positive correlation of different auxin-induced root parameters and fruit yield was also explained through the coefficient of determination (R^2). Higher value of R^2 indicated a strong linear relationship between the two parameters. R^2 value between fruit yield and the number of roots per leaf was higher (0.239, Figure 1) compared to root length (0.101; Figure 2); R^2 value between fruit yield and fresh root weight was 0.266 (Figure 3); R^2 value between fruit yield and rooted cuttings was 0.303 (Figure 4). The coefficient of determination between fruit yield and ASI value was the highest (0.419, Figure 5).

The 2 × 2 contingency table revealed that on the basis of yield, 11 out of 22 tomato genotypes were included in the above-average yielder group (AAY) and 8 out of 11 AAY genotypes had high ASI values (Table 3). In contrast, 8 out of the 11 BAY genotypes had low ASI values. Contingency chi-square value was found to be significant (4.54), and indicated that the distribution of genotypes was non-random and the HAR class had a higher frequency of high-yielding genotypes.

Auxin regulates different aspects of plant growth and development, from embryogenesis to senescence by controlling gene expression through the transcriptional regulator, namely auxin response factors (ARFs). Wu *et al.*[14]

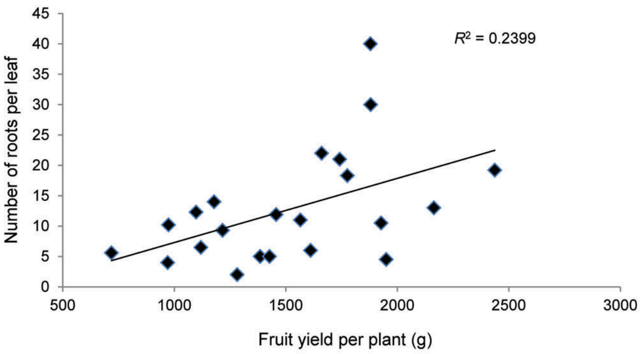

Figure 1. Relationship between fruit yield and number of roots/leaf.

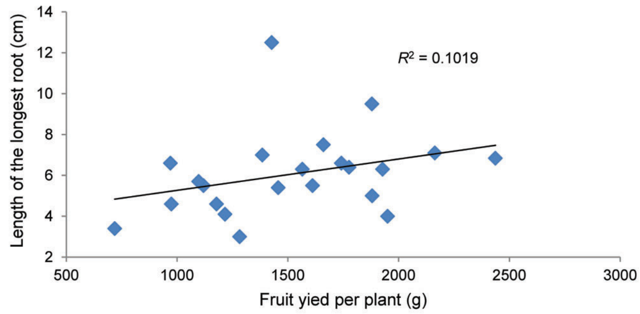

Figure 2. Relationship between fruit yield and root length.

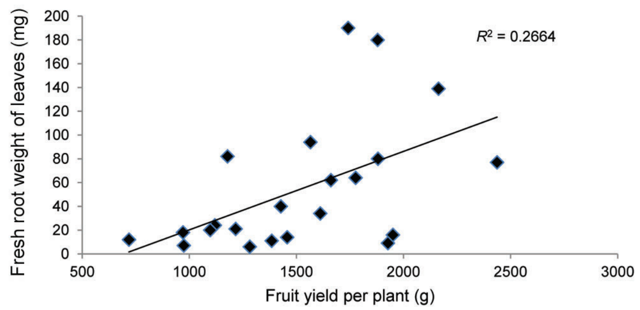

Figure 3. Relationship between fruit yield and fresh root weight.

found that tomato leaf development was co-regulated by several members of the ARF family. The functional analysis of SlARF9 (*Solanum lycopersicum* auxin response factor 9) indicated that it regulates cell division during early tomato fruit development[15]. Transgenic plants with decreased SlARF7 m-RNA levels formed seedless tomato fruits[16]. SlARF3 plays multiple roles in tomato development[17]. Quantitative PCR (qPCR) expression analysis revealed that many SlARFs were differentially expressed in tomato leaves and roots under salt, drought and flooding stress conditions, further pointing to the putative role of SlARFs in stress response. qPCR expression analysis identified some miRNA precursors as potentially involved in the regulation of their SlARF target genes in roots exposed to salt and drought stress[18].

The possible deployment of leaf culture as a cost-saving technique for different purposes in breeding new varieties is briefly discussed here with reference to tomatoes. Advances in biotechnology cannot eliminate ultimate cost intensive field experiments in evaluating yield potential and yield stability, for which leaf culture can be a useful companion if leaves are amenable to induce roots in culture. Possible uses of such leaf culture could be used evaluate yield through differential auxin response indirectly. Isolated leaves of many plants can be cultured under simple, low-cost experimental conditions. The potential of leaf culture could be better exploited in crop improvement than at present. In fact, laboratory evaluation for biotic and abiotic stress through detached leaf culture can be complementary to conventional field testing, which has several limitations besides cost. Leaf culture permits off-season testing, which is a big advantage and simultaneous evaluation of breeding material in the field and the laboratory during the crop season.

Table 3. 2 × 2 contingency table of ASI value and yield

Class	Number of genotypes	Yield class		χ^2 value
		AAY	BAY	
High auxin response	11	8	3	4.54*
Low auxin response	11	3	8	

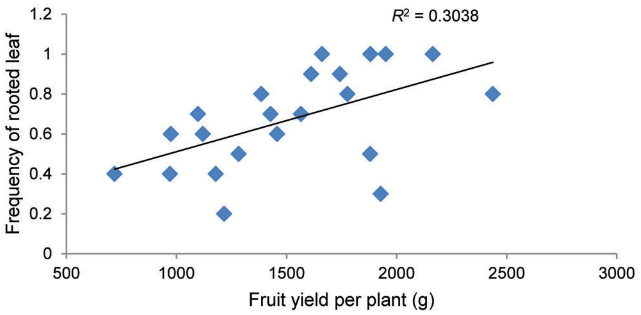

Figure 4. Relationship between fruit yield and frequency of rooted cuttings.

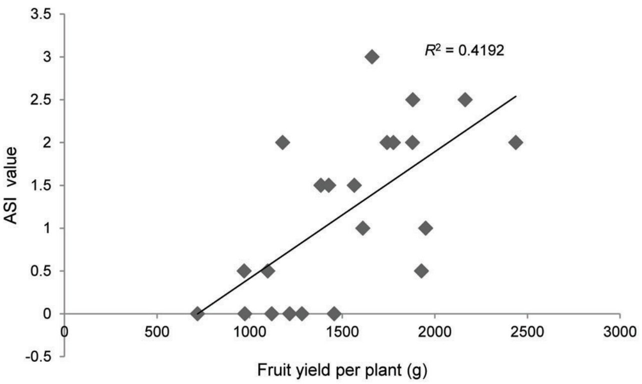

Figure 5. Relationship between fruit yield and auxin sensitivity index value.

The potential yield of tomato genotypes could be achieved only in favourable environmental conditions due to the full expression of the genetic factors. In the present study, yield was averaged over years and genotypes to obtain genotypic mean yield (grand mean yield). This was used as a yardstick to categorize high- and low-yielding genotypes. Genotypes above grand mean yield were considered high yielders and vice versa. Simple laboratory predictive techniques reduce the cost and help in preliminary and off-season evaluation of newly developed genotypes[19]. Seedling response to selected chemicals is a simple approach to indirect evaluation of yield performance[20]. Das *et al.*[21] used maleic hydrazide to screen rice (*Oriza sativa*) and ragi (*E. coracana*) genotypes for yield and adaptability. Singh *et al.*[22] used IBA for laboratory evaluation of yielding ability and adaptability in mung beans.

The present study reveals that most of the high-yielding tomato genotypes have high auxin response than low-yielding genotypes. Also, genetic variation in auxin-induced rooting response of detached leaves of tomato genotypes could be used as an indicator for screening of high-yielding tomato genotypes before going to multi-location trials.

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