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Chennem Kiran Kumar Reddy
Division of Seed Science & Technology, Indian Agricultural Research Institute, New Delhi, India

Sudheer Kumar Jain
Division of Seed Science & Technology, Indian Agricultural Research Institute, New Delhi, India

MB Arun Kumar
Division of Seed Science & Technology, Indian Agricultural Research Institute, New Delhi, India

S Gopala Krishnan
Division of Genetics, Indian Agricultural Research Institute, New Delhi, India

Awani Kumar Singh
Centre for Protected Cultivation and Technology, Indian Agricultural Research Institute, New Delhi, India

Zakir Hussain
Division of Vegetable Science, Indian Agricultural Research Institute, New Delhi, India

Correspondence
Chennem Kiran Kumar Reddy
Division of Seed Science & Technology, Indian Agricultural Research Institute, New Delhi, India

Genetic variation of seedling Trichome and root traits in four tomato hybrids and their parental lines

Chennem Kiran Kumar Reddy, Sudheer Kumar Jain, MB Arun Kumar, S Gopala Krishnan, Awani Kumar Singh and Zakir Hussain

Abstract
Density of certain types of trichomes (Type I,IV and VI) and the length of type I and IV trichomes were characterized on cotyledonary leaves and hypocotyl and root characters line length of primary root and number of secondary and tertiary roots was recorded in seedling stage in tomato to differentiate the tomato hybrids from their parental lines. Variation for Type VI trichomes was found to be significant for hybrids PH-1, PH-4 and PH-8 from one or both of their parental lines. Type I trichomes were present in only four out of ten genotypes under study *viz.*, PH-1, PH-4, Female-1 and Male-1. For root traits also, significant differences were found among the hybrids and their parental lines. For all the traits tested, the hybrids were found to be depart significantly from one or both of its parental lines with the characters studied.

Keywords: Tomato, hybrids, parental lines, genetic variation, trichomes, root characters

Introduction
Tomato (*Solanum lycopersicum* L), 2x = 24, a member of *Solanaceae*, was re-integrated into genus *Solanum* from the genus *Lycopersicum* with the revised phylogenetic classification of the family *Solanaceae*. Among the characters that are used to differentiate the tomato accessions, both qualitative and quantitative characters form the bulk of varietal characterization morphological characters. These characters are universally accepted and are considered as undisputable when they are used in a sequential fashion, Thus, these characters are the first choice in distinguishing the different varieties. Since many hybrids are continuously developed and released by both the public and private sectors and few lines are involved in transfer of their elite genes not much genetic variability can be observed in tomato. The present investigation was made to assess the genetic variation for seedling trichome and root characters between different tomato hybrids and their parental lines (Weston *et al*, 1989; Eigenbrode and Trumble, 1993) [17, 6]. Trichomes or plant hairs are commonly present in terrestrial plants and their type and density of different types of trichomes in tomato is primarily attributed to differential responses to insect pests (Carter and Snyder, 1985) [2]. These trichomes provide defense against predators by acting as a physical barrier or acting as a chemical mediated mechanism. Glandular trichomes are specialized hair like structures, found on the surface of about 30% in all vascular plants. Trichomes are store houses of specialized metabolites known for their antifungal (Mellon *et al.*, 2012) [14] and natural pesticide properties (Dayan and Duke, 2003) [5]. Their size ranges between few microns to centimeters (Payne, 1978). Trichomes of solanaceae members were studied in detail and their different classes was proposed by Luckwill (1943) [13] and revised by Channarayappa *et al.* (1992) [4]. Of the eight different types reported, four (Type I, IV, VI and VII) were glandular capitate trichomes and the rest four (Type II, III, V and VIII) are non-glandular type. In cultivated tomato species, type I, III, V and VI were abundantly distributed, whereas type VII and VIII were less abundant (Glas *et al.*, 2012) [9]. Beyond genetic control, the density of trichomes is also dependent on tissue (Kang *et al.*, 2010) [12] and environmental conditions (Wilkens *et al.*, 1996) [18].

Materials and Methods
A total of four tomato hybrids and their parental lines (Table 1) developed and released by Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi were used in the present study.

Table 1: Tomato hybrids and their parental lines used in the study

Hybrid	Female	Male
PH-1	Female-1	Male-1
PH-2	Female-2	Male-2
PH-4	Female-2	Male-1
PH-8	Female-3	Male-3

Seedling trichome characters

Experiment was conducted on seedlings raised in plug-trays, in the divisional walk-in-room germinator maintaining 25°C. Seedlings from all genotypes were raised in plug trays with single seedling in each plug for 15 days. Fully expanded cotyledonary leaves of seedlings, 15-18 days old, were selected for stomata studies (stage coincided with the emergence of apical primordial leaf). Observations were recorded on ten plants on 18-day-old seedlings. ***T***he density of different types of trichomes I, IV and VI, length of type I and IV types of trichomes were recorded using Leica stereo zoom microscope (Model DM 750) equipped with LCD light source. Photographs were taken with the Nikon DFC 295 camera attached with the microscope. Observations on trichomes were recorded by measuring the number of trichomes per millimeter from four random areas of the hypocotyl from each seedling to obtain a proper representation of trichome distribution. Pair wise comparisons (between the parental lines, hybrid with female parental line and hybrid with male parental line) were made following 't'-test proposed by Cochran and Cox approximation (Chandel, 1993) [3].

Seedling root characters under hydroponics

Hydroponic cultures were developed under glass house conditions at National Phytotron Facility, ICAR-IARI, New Delhi. The cultures were exposed to a temperature range of 16° to 22°C. Seeds from all genotypes were first germinated in a Petri dish, by placing them on two layers of blotter paper. Four-days-old germinated seedlings were transferred into the hydroponics medium. Initially, one part of Hoagland's solution was mixed with 3 parts of water and used as a medium for growing the seedlings for 2 days until the seedlings were established. Later, Hoagland's solution without dilution with water was used as medium under hydroponics until 21 days. For root characters, the observations on roots were recorded on the development of secondary roots. The development of secondary roots started on 18 days, and observations were recorded on 21-day-old seedlings. Experiment was conducted maintaining 18 plants for each genotype. Data recorded on ten plants in each genotype. Pair wise comparisons (between the parental lines, hybrid with female parental line and hybrid with male parental line) were made following 't'-test proposed by Cochran and Cox approximation (Chandel, 1993) [3].

Results

Seedling trichome characters

Significant differences were recorded among the genotypes tested for densities of type I, IV and VI trichomes on hypocotyl, and the length of type I and IV trichomes (Table 2).

Significant differences were found between the parental lines of all hybrids for density of type IV trichomes. The hybrids had trichome density on par with one of the parental lines having lower density. PH-1 had density (18.9) on par with female parental line (19.1), and statistically lower values than those of male parental line (22.1). The trichome densities in PH-2 (11.3) and PH-8 (15.0), were on par with their male parental lines (11.6 and 14.3, respectively), and statistically lower than those of their female parental lines (14.1 and 14.3, respectively). PH-4 trichome density (16.1) was statistically lower than its male (22.1) and statistically higher than its female (14.1) parental lines.

Significant differences were found for type VI trichome density in hybrids and their parental lines. PH-1 and PH-4 recorded trichome densities 2.5 and 3.0, respectively which was statistically different than both of their parental lines. Trichome density in PH-8 (2.9) was observed to be on par with its female parental line (2.4), and statistically lower than its male parental line (5.0). PH-2 with trichome density (6.7), had no significant differences among the parental lines.

For total trichome density, the hybrids PH-1 had on par density (23.9) with both of its parental lines, whereas PH-8, had density significantly lower (17.9) than both of its parental lines. PH-2 hybrid had on par density (18.1) with its male parental line (17.7), and statistically higher density than its female parental line (20.8). PH-4 recorded on par total trichome density (20.5) with its female parental line (20.8), and significantly lower density than its male parental line (24.9).

Type I trichomes were observed on four genotypes *viz.*, Female-1 (1.8), Male-1 (0.87), PH-1 (2.4) and PH-4 (1.5). PH-4 was unique from its female parental line with its type I trichomes, as its female parental line lacks type I trichomes. The type I trichomes density in PH-1 (2.4) was statistically on par with its female parental line (1.8), and statistically greater than the male parental line (0.9).

Significant differences were also found for length of type IV and type I trichomes. Hybrids PH-1 (0.251 mm) and PH-4 (0.246 mm) did not differ from their parental lines. The type IV trichome length in hybrids PH-2 (0.246 mm) and PH-8 (0.237 mm) was on par with their respective female parental lines, and statistically higher length than their male parental lines. Type I trichome length in PH-1 and PH-4 was observed to be on par with their male parental lines, and statistically lower than their female parental lines.

Seedling root characters under hydroponics

Significant differences were observed for primary root (tap root) length in the genotypes studied. The hybrids were observed to depart significantly from one or both the parental lines. PH-1 (11.2 cm) and PH-8 (14.6 cm) were statistically different from both the parental lines; and in case of PH-2 (10.5 cm) and PH-4 (16.4 cm) from corresponding female parental lines (Table 3).

With respect to number of first order roots (secondary roots), their number in hybrid was on par with the female parental line, and significantly lower than the male parental line in PH-1. The primary root length in PH-2, PH-4 and PH-8 was found to be on par with their male parental line, and statistically greater than the female parental line.

For number of second order root (tertiary roots), the hybrids PH-1, PH-2 and PH-4 had significantly higher root number than both their parental lines, whereas in PH-8 it was on par with male parental line (Table 3).

Table 2: Hypocotyl trichome characters in tomato hybrids and their parental lines

Genotype	Total number of trichomes	Number of trichome			Length of trichome (mm)	
		Type IV	Type VI	Type I	Type IV	Type I
Female-1	25.2 ± 0.9a	19.1 ± 1.0a	4.3 ± 0.4c	1.8 ± 0.3a	0.255 ± 0.01a	0.360 ± 0.02a
PH-1	23.9 ± 0.6a	18.9 ± 0.6a	2.5 ± 0.4b	2.4 ± 0.4a	0.251 ± 0.01ab	0.337 ± 0.01b
Male-1	24.9 ± 0.6a	22.1 ± 0.6b	1.9 ± 0.2a	1.0 ± 0.2 b	0.245 ± 0.01b	0.333 ± 0.01b

Genotype	Total number of trichomes	Number of trichome			Length of trichome (mm)	
		Type IV	Type VI	Type I	Type IV	Type I
Female-2	20.8 ± 0.5a	14.1 ± 0.6a	6.7 ± 0.5a	0	0.241 ± 0.01a	NA
PH-2	18.1 ± 0.5b	11.3 ± 0.6b	6.7 ± 0.4a	0	0.246 ± 0.01a	NA
Male-2	17.7 ± 0.5b	11.6 ± 0.6b	6.2 ± 0.4a	0	0.223 ± 0.01b	NA

Genotype	Total number of trichomes	Number of trichome			Length of trichome (mm)	
		Type IV	Type VI	Type I	Type IV	Type I
Female-2	20.8 ± 0.5a	14.1 ± 0.6a	6.7 ± 0.5c	0	0.241 ± 0.01a	NA
PH-4	20.5 ± 0.6a	16.1 ± 0.7b	3.0 ± 0.3b	1.5 ± 0.2a	0.246 ± 0.01a	0.327 ± 0.01a
Male-1	24.9 ± 0.6b	22.1 ± 0.6c	1.9 ± 0.2a	1.0 ± 0.2a	0.245 ± 0.01a	0.333 ± 0.01a

Genotype	Total number of trichomes	Number of trichome			Length of trichome (mm)	
		Type IV	Type VI	Type I	Type IV	Type I
Female-3	20.4 ± 0.4a	17.9 ± 0.4a	2.4 ± 0.4a	0	0.236 ± 0.01ab	NA
PH-8	17.9 ± 0.5b	15.0 ± 0.4b	2.9 ± 0.4a	0	0.237 ± 0.01b	NA
Male-3	19.3 ± 0.6a	14.3 ± 0.6b	5.0 ± 0.4b	0	0.233 ± 0.01a	NA

Where, values are mean values ± standard error, Values with same letters are statistically on par; and with different letters are significantly different
NA: Not applicable 0: Not found

Table 3: Root parameters in tomato hybrids and their parental lines

Genotype	Primary root length (cm)	Number of root	
		secondary	tertiary
Female-1	9.0 ± 0.3a	26.0 ± 1.0a	2.3 ± 0.3a
PH-1	11.2 ± 0.4b	26.1 ± 0.8a	5.8 ± 0.9b
Male-1	15.5 ± 0.7c	32.3 ± 1.8b	3.8 ± 0.3c

Genotype	Primary root length (cm)	Number of root	
		secondary	tertiary
Female-2	12.0 ± 0.3a	25.9 ± 1.0a	0.8 ± 0.1a
PH-2	10.5 ± 0.4b	30.1 ± 1.2b	2.2 ± 0.3b
Male-2	10.3 ± 0.3b	31.7 ± 1.4b	1.6 ± 0.2c

Genotype	Primary root length (cm)	Number of root	
		secondary	tertiary
Female-2	12.0 ± 0.3a	25.9 ± 1.0a	0.8 ± 0.1a
PH-4	16.4 ± 0.6b	30.2 ± 1.7b	5.1 ± 0.3c
Male-1	15.5 ± 0.7b	32.3 ± 1.8b	3.8 ± 0.3b

Genotype	Primary root length (cm)	Number of root	
		secondary	tertiary
Female-3	12.1 ± 0.3b	28.0 ± 2.1a	0.2 ± 0.1a
PH-8	14.6 ± 0.6c	35.5 ± 1.3b	2.6 ± 0.3b
Male-3	11.0 ± 0.4a	33.5 ± 1.2b	1.8 ± 0.3b

Where, values are mean values ± standard error, Values with same letters are statistically on par; and with different letters are significantly different

Discussion

Trichomes are hair like appendages on the plant surface of many angiosperms primarily involved in plant defense mechanism. Irrespective of genetic control of trichomes distribution, their density also depends on tissue (Kang *et al.*, 2010) [12] and environmental conditions (Wilkens *et al.*, 1996) [18]. The seedlings were grown under uniform conditions maintaining uniform plant density to avoid the effect of micro-climate. The genotypes differed significantly for type-IV and VI trichome densities. In tomato, high density of type IV and VI trichomes is a recessive trait (Freitas *et al.*, 2002) [8]. In accordance with this, the hybrids recorded lower trichome density which was on par with the values recorded in one of the parental lines. Until date, this character has been primarily used for its correlation with pest resistance (Snyder and Carter, 1984; Frietas *et al.*, 2002; Wang *et al.*, 2001; Hawthorne *et al.*, 1992) [8, 16, 4]. In the present study, the utility of this hypocotyl trichome trait in distinguishing the hybrid from its parental lines was established.

Root characters like primary root length, number of secondary root and tertiary roots were used to distinguish the cultivars for their ability to withstand drought in rice (Elkhoby *et al.*, 2014, Abd-Allah *et al.*, 2013) [7, 1] and in tomato (Indu *et al.*, 2009) [11].

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