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RESEARCH ARTICLE

Genotype trait analysis to identify potential resistant types to root knot nematode in tomato (*Solanum lycopersicum* L.)

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Abstract

Six F_1 hybrids were tested for nematode resistance along with their parents and commercial cultivars for two seasons and the pooled mean results were analysed to explore the genetic potential of traits linked to specific genotypes. Nine quantitative, four qualitative and six physiological parameters were analyzed to identify superior hybrids through statistical models *viz*., analysis of variance, GT biplot and Wards clustering. The hybrids Hisar Arun×HN2 (susceptible), Arka Abha×HN2 and LE 812×HN2 (resistant) were found to be superior for yield per plant under stressed conditions. Higher plant height was observed in IIHR 2868 (86.21cm), primary branch in Arka Abha×HN2 (5.68) and shorter root length in Hisar Lalith (16 cm). Resistant hybrids exhibited earlier flowering *i.e*., 25 days . The susceptible genotypes expressed lower fruit number (17-20 fruits) on the contrary resistant hybrids had more than 20 fruits. The yield ranged between 667 g in CLN2123A and 1189 g in Hisar Arun×HN2. Under stressed conditions, the resistant hybrids Arka Abha×HN2 and LE 812×HN2 produced yields of 1169 g and 1153 g respectively. Genotype Trait biplot revealed that the PC_1 and PC_2 had contributed 70 % to the total variance and positive contributions to parents and hybrids were capped. The hybrids LE 812×HN2 and Arka Abha×HN2 can be well utilized in root knot nematode infested fields. The contribution of parents and its hybrids, associated traits and their interrelationships provide new dimension for the breeders to select trait specific parents and hybrids for crop improvement programs.

Keywords

GT biplot; hybrid; peroxidase; tomato; ward's cluster; yield

Introduction

Tomato (*Solanum lycopersicum* L.) is a widely grown versatile diploid self-pollinated vegetable crop (1) belonging to Solanaceae family and an important vegetable crop throughout the world (2) with the primary centre of origin in the Peru and Ecuador region. Tomato is a rich source of minerals and nutrients (3) and hence, it is considered as the "protective foods" and "poor man's orange" because of its nutritive value (4). Crop improvement in tomato is over a century old and the crave for development of new cultivars for the human race is interminable.

Biotic stresses cause enormous loss in tomato and are therefore, have been studied for centuries. One among these stresses is the nematodes. Among the different *Meloidogyne* spp*., viz.*, *M. incognita, M. javanica, M. arenaria, M. ethiopica, M. hapla, M. acronea,* (5), *Meloidogyne incognita* (RKN's) causes huge production loss of tomato in tropical and subtropical countries like India (6). Globally RKN lead to 26 % to 73 % reduction in tomato yield, worth about $125 billion annually (7). RKN's are generally endoparasites (8), show parthenogenetic reproduction and can survive for two or more years even in the absence of any host (9). The general symptoms of nematode injury on tomato are stunted growth (10) accompanied with reduction in yield (11). Hence, the need of the hour is to improve the resistance of the plants against nematode infestation.

To ensure the development of resistance in plants, many techniques have been employed previously. However, a hybridization programme can be considered as a best alternative for the development of varieties with both yield potential and quality under stress conditions. Determination of diversity and genetic relationships in breeding materials is an inevitable process in crop improvement strategies (12). Genotype-Trait (GT) biplot technique (13) can be used to assess the adaptability of wild and cultivated tomato hybrids, as well as the NaCl tolerance of tomato genotypes at the seedling stage (14). GT biplot has helped breeders to investigate data of various traits at once to improve indirect selection of parental lines, unlike most univariate tools that explore traits in the dataset separately (15). GT Biplots are 2D and require replicated data to visualize the population distance or associated traits in the group. On the other side, the pooled analysis contains mean data and to explore the contribution of parents and hybrids to total variance requires alternate statistical methods.

The primary objective of this study is to select diverse parents and hybrids for nematode resistance coupled with the degree of associated traits for indirect selection by the breeders at an earlier stage.

Materials and Methods

The present investigation was conducted at the Department of Vegetable Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, during 2018-19 in two Seasons, which is situated at 11°N Latitude, 77° E Longitude and at an altitude of 426.26 m above MSL. The details of the parents and hybrids of tomato is presented in Table 1.

Table 1. Details of Parents and hybrids of tomato in the experiment

Parents	Accessions	Origin	Hybrids	Cross combinations
P1	IC 249503	India	H1	IC 249503 × HN2
P2	CLN 2123A	Taiwan	H2	CLN 2123A × HN2
P3	Hisar Arun	India	H3	Hisar Arun × HN2
P4	LE 812	India	H4	LE 812 × HN2
P5	Arka Abha	India	H5	LE 812 × IIHR 2868
P6	HN 2	India	H6	Arka Abha × HN2
P7	IIHR 2868	India	H7	Hisar Lalith (check)
P8	PKM 1 (check)	India		

The experiment was conducted in a completely randomized design with five replications. All the experimental materials were tested in two seasons *viz*., winter and summer season and the pooled analysed data of both experiments are utilized for this study.

Biometric parameters

The plant height was measured from ground level to the tip of the plant at the time of the final harvest and the mean was figured and expressed in centimetres. The number of branches on the main stem were recorded at the time of first harvest and the mean values were computed. Earliness in flowering was calculated by recording number of days taken from the date of transplanting to the first flowering of plant. The total number of fruits per plant during each harvest was counted and expressed in number of fruits per plant after completion of harvesting. The weight of single fruit at each harvest was noted in five randomly selected fruits and the mean of all harvests was noted and expressed in grams. The weight of all the red ripe fruits from each harvest was recorded and the total yield per plant was worked out by adding yield of all harvests and was expressed in grams per plant. Each fruit was dissected transversely and the number of locules was counted from the five fruits and averaged. The length of root was measured from the base of the plant to the tip of the roots in three randomly selected plants in each replication and the mean was expressed in centimetre. Then the roots were dried in hot air oven at 60° C for 72 hours and the mean was expressed as root dry weight in gram.

Quality parameters

S.	Parameters	Methodology
1	TSS, Acidity and Lycopene	(16)
2	Ascorbic acid	(17)

Physiological parameters

S. No	Parameters	Methodology reference
1	Chlorophyll content	(18)
2	Total Phenols	(19)
3	Peroxidase and PPO	(20)
4	IAA oxidase	(21)
5	Acid Phosphatase	(22)

Statistical Analysis

The recorded observations were subjected to the statistical analysis. The pooled data were analysed by analysis of variance and tests of significance at $p< 0.05$ for each trait using a Linear model and DNMRT for grouping in RStudio ver. 2024.04.1. The Genotype-Trait (GT) biplot through principal component analysis (PCA) and Ward's hierarchical cluster analysis were utilized for the study. PCA can exhibit the contribution of associate trait to the total variations observed in a population. The parameters with the highest selective ability were based on the degree of positive association, while Euclidean distance can show the

level of association among samples. The phylogenetic relationships between population and traits were analysed by Wards method in PAST 4.03 software.

Results and Discussion

Performance of parents and hybrids

Vegetative parameters

The vegetative parameters *viz*., plant height, number of primary branches, root length and weight varied significantly across the parents and hybrids (Table 2). The genotype IIHR 2868 had significantly registered taller plants (86.21 cm) and CLN 2123A recorded dwarf (72.43 cm) in the case of parents, while in hybrids Arka Abha×HN2 recorded tall plants (85.87 cm) and CLN 2123A×HN2 was dwarf (78.93 cm), indicating the expression of hybrid vigour for this trait (23). Conversely, taller plants (137.43 cm) in EC-157568×Arka Vikas (24). Higher number of primary branches per plant was recorded in Arka Abha (5.05) and lower in IC 249503 (3.61), whereas in hybrids Arka Abha×HN2, registered the highest (5.68) and Hisar Arun×HN2 recorded the lowest number of primary branches (4.92). In general, the hybrids registered higher number of primary branches than the parents. Formation of lateral shoots increases the stem numbers per plant, which has a direct effect on the terminal flower production in tomato plants (25).

stressed conditions. Root-knot nematodes (RKN) are obligate endoparasites that severely damage the host root system, which enter through the lenticels or any damaged root parts and multiply intercellularly (Fig. 7) to establish their feeding site and disrupt the vascular tissue (26).

After transplanting at an age of 25 days from sowing, earlier flowering was recorded in LE 812 (25.82 days), whereas late in IC 249503 (32.33 days). In the case of hybrids, least number of days taken to first flower was recorded in LE 812×HN2 (25.61 days) followed by Arka Abha×HN2 (25.85 days) and late in IC 249503×HN2 as 30.01 days after transplanting. All the resistant hybrids recorded earlier flowering compared to their counterparts (Table 3), which is an indication of stress escape mechanism (27). In tomato, the stress affects the plant at differentiation stages *viz*., vegetative phase, at flowering and after flowering. The stress effects after flowering had negligible effect on the yield as compared to other two stages. In the infested plants, the nutrient and water uptake are substantially reduced which results in altered plant growth (28).

A higher number (23.02) of fruits per plant was recorded in IIHR 2868 followed by Arka Abha (22.69) and a lower fruit count was observed in CLN 2123A (17.05). In the case of hybrids, the highest was recorded in Arka Abha×HN2 (27.02) followed by IC 249503×HN2 (25.23) and the

Table 2. Vegetative parameters of parents and crosses

Parents & Hybrids	Plant Height (cm)	Primary branch (Nos.)	Root length (cm)	Root weight (cm)
IC 249503	74.31±1.44^{cd}	3.61±0.52^{f}	10.95±1.33^{g}	3.55±0.02^{ab}
CLN 2123A	72.43±0.65^{d}	4.19±0.09^{ef}	17.71±2.33^{def}	2.49±0.06^{cd}
Hisar Arun	75.84±2.67^{bcd}	4.48±0.25^{de}	19.17±2.89^{de}	2.30±0.08^{d}
LE 812	78.65±0.51^{bc}	4.61±0.13^{cde}	17.43±2.04^{def}	3.00±0.22^{bc}
Arka Abha	78.95±0.68^{bc}	5.05±0.08^{abcd}	13.96±0.76^{fg}	3.42±0.70^{ab}
HN 2	76.17±6.05^{bcd}	5.03±0.05^{abcd}	25.79±6.17^{bc}	1.36±0.07^{f}
IIHR 2868	86.21±4.24^{a}	4.99±0.18^{abcd}	21.69±1.32^{cd}	1.93±0.55^{def}
IC 249503×HN2	80.14±0.21^{b}	5.58± 0.37^{a}	25.99±2.64^{abc}	1.57±0.20^{ef}
CLN 2123A×HN2	78.93±0.39^{bc}	5.33± 0.16^{abc}	25.18±3.54^{bc}	1.97±0.21^{def}
Hisar Arun×HN2	78.06±1.61^{bcd}	4.92± 0.51^{abcde}	24.53±3.39^{bc}	2.23±0.36^{d}
LE 812×HN2	80.29±0.05^{b}	5.43± 0.24^{ab}	26.52±2.60^{ab}	1.49±0.15^{f}
LE 812×IIHR 2868	78.14±1.16^{bc}	5.23± 0.16^{abcd}	24.44±2.88^{bc}	2.20±0.27^{de}
Arka Abha×HN2	85.87±0.16^{a}	5.68± 0.25^{a}	27.59±2.28^{ab}	1.35±0.01^{f}
Hisar Lalith	74.61±1.94^{bcd}	4.55±0.27^{cde}	16.00±7.80^{ef}	4.01±0.40^{a}
PKM 1	76.43±3.13^{bcd}	4.72±0.99^{bcde}	30.37±5.15^{a}	1.96±0.25^{def}

± SD; P(0.05).

The root length ranged from 30.37 cm (PKM1) to 10.95 cm (IC 249503) in parents, while in hybrids longer (27.59 cm) in Arka Abha×HN2. The length of the root is an indicator of the variety adaptability to a given environment. In this study, the check PKM1 is the predominant cultivar among the farmers of this region. The root dry weight ranged between 1.36 g (HN2) to 3.55 g (IC 249503) in parents. In case of hybrids, the lowest root weight was recorded in Arka Abha×HN2 (1.35 g) and significantly the highest in the commercially nematode resistance check, Hisar Lalith (4.01g) implying the genotypic effect over the lowest in Hisar Arun×HN2 (21.81). These results were in accordance with the findings of other workers (29,30). The maximum fruit weight was recorded in Hisar Arun (48.23) followed by LE 812 (40.96) and lowest in HN2 (33.98). While in hybrids, the highest fruit number was recorded in Hisar Arun×HN2 (54.35) followed by LE 812×HN2 (47.41) and the lowest in CLN 2123A×HN2 (39.83). The lowest number of locules per fruit was observed in IIHR 2868 (2.94) whereas highest in IC 249503 (5.04) in the case of parents. In hybrids, the lowest number of locules was observed in CLN 2123A×HN2 (2.80) followed by LE 812×HN2 (3.34) and

Table 3. Reproductive traits of parents and hybrids

Parents & Hybrids	Earliness in flowering (Days)	Fruit number/ plant (Nos.)	Individual fruit weight (g)	No of Locules per fruit (Nos.)
IC 249503	32.33±0.80^{a}	19.31±2.73^{fgh}	37.38±3.42^{d}	5.04±0.08^{a}
CLN 2123A	29.44±0.07^{cde}	17.05±3.92^{h}	38.87±2.73^{bcd}	3.37±0.21^{defg}
Hisar Arun	28.16±0.26^{ef}	19.85±2.56^{efgh}	48.23±8.56^{ab}	4.95±0.11^{a}
LE 812	25.82±0.70^{g}	20.86±2.26^{defg}	40.96±1.61^{bcd}	3.25±0.05^{defg}
Arka Abha	26.98±0.89^{fg}	22.69±2.64^{bcde}	39.25±2.73^{bcd}	3.82±0.34^{bcde}
HN 2	29.40±0.11^{cde}	22.09±3.61^{cdef}	33.98±5.36^{d}	4.19±0.25^{b}
IIHR 2868	31.18±1.52^{ab}	23.02±3.11^{bcd}	36.73±2.81^{d}	2.94±0.82^{fg}
IC 249503×HN2	30.01±0.97^{bcd}	25.23±5.38^{ab}	40.80±1.77^{bcd}	3.49±0.10^{cdef}
CLN 2123A×HN2	28.88±0.33^{de}	23.47± 5.30^{bcd}	39.83±2.37^{bcd}	2.80±0.23^{g}
Hisar Arun×HN2	25.84±0.73^{g}	21.81±3.39^{cdef}	54.35±1.72^{a}	4.96±0.23^{a}
LE 812×HN2	25.61±0.57^{g}	24.29±4.79^{bc}	47.41±0.56^{abc}	3.18±0.08^{efg}
LE 812×IIHR 2868	30.89±0.52^{abc}	22.78±4.50^{bcd}	42.27±1.12^{bcd}	3.85±0.01^{bcd}
Arka Abha×HN2	25.85±0.69^{g}	27.02±4.81^{a}	43.09±1.36^{bcd}	3.86±0.15^{bcd}
Hisar Lalith	27.9±0.5^{9ef}	23.69±4.94^{bcd}	38.35±3.84^{cd}	4.89±0.05^{a}
PKM 1	28.92±0.41^{de}	18.88±1.53^{gh}	38.53±9.38^{bcd}	4.08±0.03^{bc}

± SD; P(0.05).

highest in Arka Abha×HN2 (4.38). The locules in tomatoes vary from two to ten (31, 32).

The highest yield per plant was recorded in Hisar Arun (971.24 g) followed by Arka Abha (892.10 g), LE 812 (855.19 g) and the lowest in CLN 2123A (667.56 g) in parents, while in hybrids (Fig.1), the highest yield per plant was recorded in Hisar Arun×HN2 (1189.0 g) followed by Arka Abha × HN2 (1169.27 g), LE 812×HN2 (1153.74 g) and

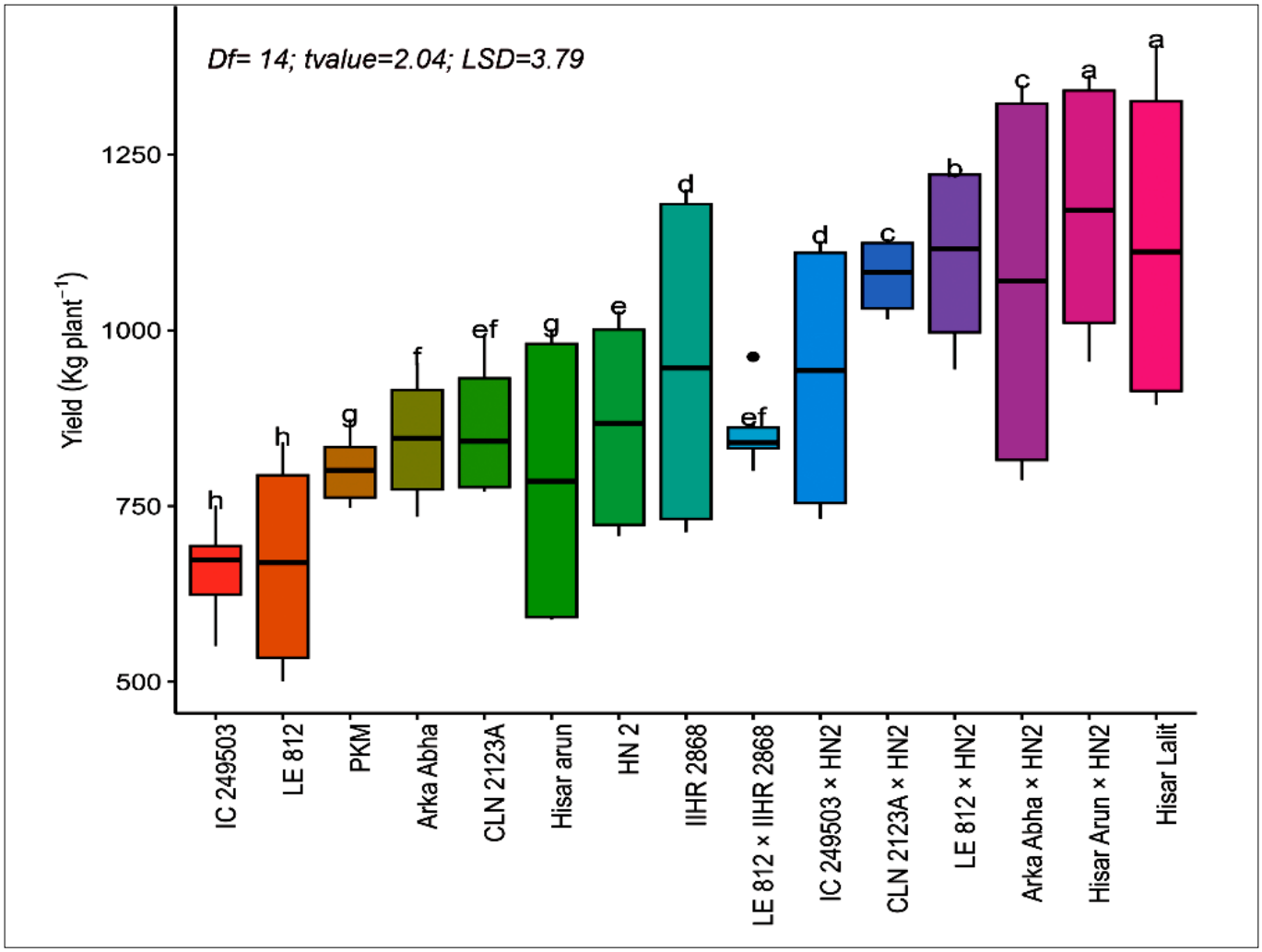

Fig.1. Variation in yield of tomato.

the lowest in CLN 2123A×HN2 (938.71 g) in pooled analysis. Even though Hisar Arun×HN2 was regarded as susceptible to RKN, this hybrid had out yielded other hybrids, which indicate the genetic potential of the cross combination. Hence, its high time to study the trait base genotype to help the breeders to identify superior crosses rather than relying on the observable values.

Physiological parameters

The highest total phenol content (Table 4) was recorded in HN2 (116.53 µg/g) followed by IIHR 2868 (112.72 µg/g) and lowest in IC 249503 (60.13 µg/g) whereas in hybrids, the highest was recorded in Hisar Lalith (124.5 µg/g), Arka Abha×HN2 (117.45 µg/g) followed by LE 812×HN2 (114.99 µg/g) and lowest in LE 812×IIHR 2868 as 100.20 µg/g. The total phenol content ranged from 1.89 mg/100 g to 3.28 mg/100 g (33). Higher the phenol content, higher the degree of nematode resistance. It was in line with the findings of (34) in which the resistant male parent had registered higher level of peroxidase and polyphenol oxidase enzyme compared to its susceptible check and the resistance was transferred through hybridization.

Table 4. Physiological parameters of tomato parents and hybrids

Parents & Hybrids	Total phenol	Polyphenol oxidase	Peroxidase	IAA oxidase	Acid Phosphatase	Total chlorophyll content
IC 249503	60.13±7.79^{ef}	1.89±0.20^{e}	1.49±0.01^{ef}	33.26±9.89^{b}	72.10±0.71^{g}	1.36±0.13^{fg}
CLN 2123A	74.10±4.75^{d}	2.26±0.09^{e}	1.69±0.03^{e}	42.92±6.14^{b}	75.01±2.12^{f}	1.48±0.14^{ef}
Hisar Arun	71.59±5.23^{d}	2.28±0.21^{e}	1.71±0.22^{e}	43.66±8.3^{b}	75.73±2.12^{f}	1.66±0.10^{de}
LE 812	68.26±5.32^{de}	2.07±0.01^{e}	1.33±0.07^{ef}	38.59±8.78^{b}	69.76±1.41^{h}	1.50±0.05^{ef}
Arka Abha	60.31±9.88^{ef}	2.18±0.08^{e}	1.41±0.35^{ef}	36.10±17.51^{b}	73.70±0.71^{fg}	1.52±0.21^{ef}
HN 2	116.53±1.80^{ab}	3.50±0.18^{abc}	3.25±0.04^{ab}	75.87±5.16^{a}	82.81±2.12^{abc}	2.07±0.07^{ab}
IIHR 2868	112.72±1.44^{b}	3.47±0.20^{abc}	2.59±0.37^{d}	73.92±4.02^{a}	81.12±2.12^{cd}	1.94±0.06^{bc}
IC 249503×HN2	111.94±0.96^{b}	3.33±0.23^{bcd}	2.78±0.08^{bcd}	79.16±3.11^{a}	79.82±2.12^{de}	1.77±0.07^{cd}
CLN 2123A×HN2	101.04±0.19^{c}	3.13±0.03^{cd}	2.52±0.24^{d}	77.33±2.26^{a}	79.77±0.78^{de}	1.51±0.02^{ef}
Hisar Arun×HN2	101.67±4.45^{c}	2.97±0.08^{d}	2.31±0.46^{d}	72.57±0.64^{a}	74.89±5.05^{f}	1.61±0.11^{de}
LE 812×HN2	114.99±2.81^{ab}	3.50±0.40^{abc}	3.13±0.19^{abc}	79.85± 2.28^{a}	82.15±1.56^{bcd}	2.08±0.10^{ab}
LE 812×IIHR 2868	100.20±3.45^{c}	3.01±0.08^{d}	2.73±0.16^{cd}	75.42±0.43^{a}	78.44±1.10^{e}	1.35±0.14^{fg}
Arka Abha×HN2	117.45±1.17^{ab}	3.79±0.08^{a}	3.24±0.07^{ab}	81.58±2.92^{a}	83.90±1.89^{ab}	2.18±0.01^{a}
Hisar Lalith	124.5±1.44^{a}	3.62±0.11^{ab}	3.41±0.06^{a}	86.58±1.49^{a}	85.13±1.56^{a}	2.21±0.02^{a}
PKM 1	54.43±10.14^{f}	1.17±0.28^{f}	1.12±0.16^{f}	36.85±6.75^{b}	67.90±1.91^{h}	1.21±0.07^{g}

± SD; P(0.05).

Total chlorophyll content was observed to be higher in HN2 (2.07 mg/g) and IC 249503 (1.36 mg/g) recorded lower values. In case of hybrids, significantly higher chlorophyll content was observed in Hisar Lalith (2.21mg/g), Arka Abha×HN2 (2.18 mg/g) and lower in LE 812×IIHR 2868 (1.35 mg/g).

HN2 parent recorded the highest peroxidase activity (3.25 ΔA/g/min) whereas the lowest was recorded in LE 812 (1.33 ΔA/g/min). In case of hybrids, the highest peroxidase activity was observed in Arka Abha×HN2 (3.24 ΔA/g/min) while the lowest in Hisar Arun×HN2 (2.30 ΔA/g/min). Among the parents, the highest polyphenol oxidase activity was observed in HN2 (3.50 ΔA/g/min) and the lowest in IC 249503 (1.89 ΔA/g/min). Whereas in hybrids, Arka Abha×HN2 (3.78 ΔA/g/min) was followed by LE 812×HN2 (3.50 ΔA/g/min) registered highest activity. While the lowest activity was registered in Hisar Arun×HN2 (2.97 ΔA/g/min). The polyphenol oxidases are involved in production of phytoalexins through oxidation of phenolic compounds under stressed conditions (35). With reference to IAA oxidase activity, the parent HN2 recorded the highest activity (75.87 µg/100mg) while IAA oxidase activity was the lowest in Arka Abha (36.09 ΔA/g/min). In case of hybrids, highest was recorded in Hisar Lalith (86.58 ΔA/g/min), Arka Abha×HN2 (81.58 ΔA/g/min) followed by LE 812×HN2 (79.85 ΔA/g/min) and lowest in Hisar Arun×HN2 (72.57 ΔA/g/min). Acid phosphatase activity recorded higher in HN2 (82.81 *m* moles of p-nitrophenol/min /mg) followed by IIHR 2868 (79.62 *m* moles of p-nitrophenol/min /mg) and lowest activity in LE 812 (69.76 *m* moles of p-nitrophenol/min /mg) among the parents. In case of hybrids, higher acid phosphatase activity was recorded in Hisar Lalith (85.13 *m* moles of p-nitrophenol/min /mg), Arka Abha×HN2 (83.90 *m* moles of p-nitrophenol/min /mg) followed by LE 812×HN2 (82.15 *m* moles of p-nitrophenol/min /mg) and lowest in Hisar Arun×HN2 (74.89 *m* moles of p-nitrophenol/min /mg).

Quality parameters

The highest TSS was recorded in PKM1 (5.38 °Brix) and the lowest in IIHR 2868 (4.92 °Brix) in parents while in hybrids Arka Abha×HN2 (5.29 °Brix) which was higher than the better parent. The highest acidity content was observed in IIHR 2868 (0.61 %) followed by HN2 (0.60 %) and the lowest in Hisar Arun (0.48 %) in case of parents (Table 5). In hybrids, higher was recorded in Hisar Lalith (0.61), Arka Abha×HN2 (0.59 %) followed by LE 812×HN2 (0.56 %) and lower in Hisar Arun×HN2 (0.49 %). Arka Abha recorded the highest value for ascorbic acid (33.20 mg/100g), followed by LE 812 (32.40 mg/g) and lowest in HN2 (25.78 mg/g) while in hybrids, the highest value was recorded in Arka Abha×HN2 (35.94 mg/g) followed by LE 812×HN2 (34.74 mg/g) and the lowest in LE 812×IIHR 2868 (31.64 mg/g).

Table 5. Variation in quality parameters of tomato for root knot nematode resistance

Parents & Hybrids	TSS (°Brix)	Acidity (%)	Ascorbic acid (%)	Lycopene (mg/100g)	RKN reaction
IC 249503	4.95±0.30^{ab}	0.51±0.04^{defg}	28.13±1.57^{d}	4.13±0.01^{h}	Highly susceptible
CLN 2123A	5.02±0.26^{ab}	0.51±0.01^{efg}	32.15±1.15^{bc}	4.42±0.15^{fgh}	Susceptible
Hisar Arun	5.08±0.37^{ab}	0.48±0.02^{g}	31.19±2.52^{c}	4.43±0.61^{fgh}	Susceptible
LE 812	5.20±0.17^{ab}	0.52±0.02^{cdef}	32.40±0.98^{bc}	4.71±0.12^{cdef}	Highly susceptible
Arka Abha	5.03±0.10^{ab}	0.56±0.02^{bcd}	33.20±0.59^{abc}	4.53±0.01^{defgh}	Highly susceptible
HN 2	4.96±0.33^{ab}	0.60±0.03^{a}	25.78±1.56^{d}	4.81±0.21^{cdef}	Resistant
IIHR 2868	4.92±0.21^{b}	0.61±0.01^{a}	32.11±1.11^{bc}	5.10±0.01^{bc}	Resistant
IC 249503×HN2	5.15±0.17^{ab}	0.52±0.01^{cdef}	34.52±0.86^{ab}	4.94±0.22^{bcd}	Moderately resistant
CLN 2123A×HN2	5.09±0.11^{ab}	0.52±0.01^{cdef}	32.82±0.23^{abc}	4.61±0.05^{defg}	Moderately resistant
Hisar Arun×HN2	5.04±0.28^{ab}	0.49±0.01^{fg}	33.60±0.67^{abc}	4.48±0.12^{efgh}	Susceptible
LE 812×HN2	5.21±0.13^{ab}	0.56±0.01^{bc}	34.74±0.31^{ab}	5.34±0.04^{ab}	Resistant
LE 812×IIHR 2868	5.12±0.11^{ab}	0.51±0.01^{efg}	31.64±0.42^{bc}	4.16±0.09^{gh}	Susceptible
Arka Abha×HN2	5.29±0.11^{ab}	0.59±0.01^{ab}	35.94±1.36^{a}	5.63±0.05^{a}	Resistant
Hisar Lalith	5.02±0.74^{ab}	0.61±0.03^{a}	32.42±1.74^{bc}	4.91±0.16^{bcde}	Resistant
PKM 1	5.38±0.27^{a}	0.55±0.00^{cde}	31.20±1.64^{c}	4.91±0.01^{bcde}	Highly susceptible

± SD; P(0.05).

The results are in confirmation with the findings of (36,37,38). Lycopene content was higher in IIHR 2868 (5.10 mg/100g) and lower in IC 249503 (4.13 mg/100g) in parents while in hybrids, the highest was observed in Arka Abha×HN2 (5.63 mg/100g) followed by LE 812×HN2 (5.34 mg/100g) and the lowest in LE 812×IIHR 2868 (4.16 mg/100g). This was in conformity with the findings of (39)

GT biplot analysis

Principal component analysis was used to exploit the source of actual variation and the total contribution of the observed characteristics. As a method of multivariate analysis, it had transformed the initial variables into a limited number of correlated new variables. The Principal Component Analysis (PCA) was analyzed using 9 parents and 6 F_1 hybrids which include phenotypic, physiological and quality parameters at 95% confidence level. The utilization of hybrids in this study is to identify superior hybrids at F_1 stage and it can be used in further breeding programs. The percent variation based on correlation revealed that the clusters PC_1 (53.49 %), PC_2 (17.28 %) and PC_3 (9.49 %) contributed the major share for the variation (81.83%) which could be observe from Scree plot (Fig.2). The Eigen value more than 1 showed at least 10% variation (40) and it is measure of best representative of system attribute in principal components (41, 42). Hence the 2D GT Biplots were analysed for PC_1 and PC_2 which contributed 70% of the total variation. Contribution of the first two components to total variance as well as the characteristics which were crucial for components to be ascertained.

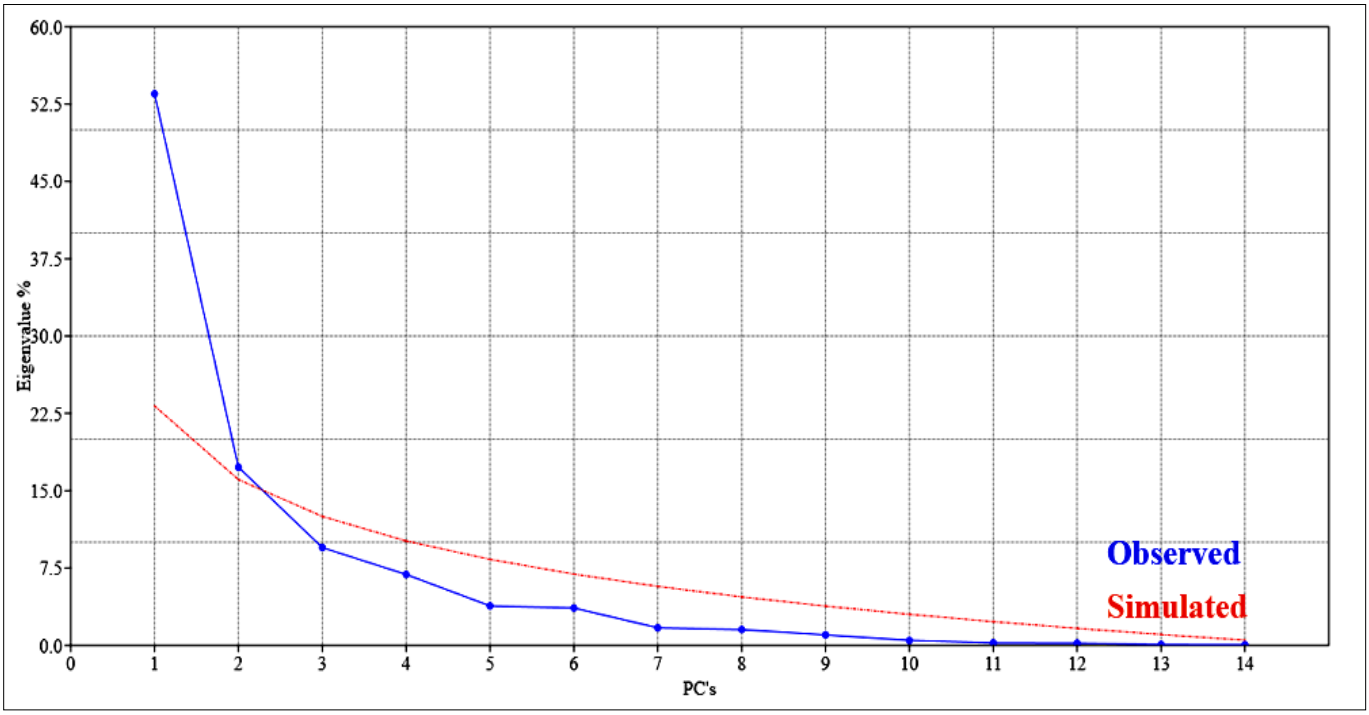

Fig.2. Scree plot of principal components.

In this study, the first component (>0.5 correlation) accounts majority of the traits *viz*., plant height, number of primary branches, number of fruits per plant, individual fruit weight, root length and all the physiological parameters, lycopene and yield (Fig 3) registered positive contributions which indicate the selection pressure to be applied while selection of the hybrids.

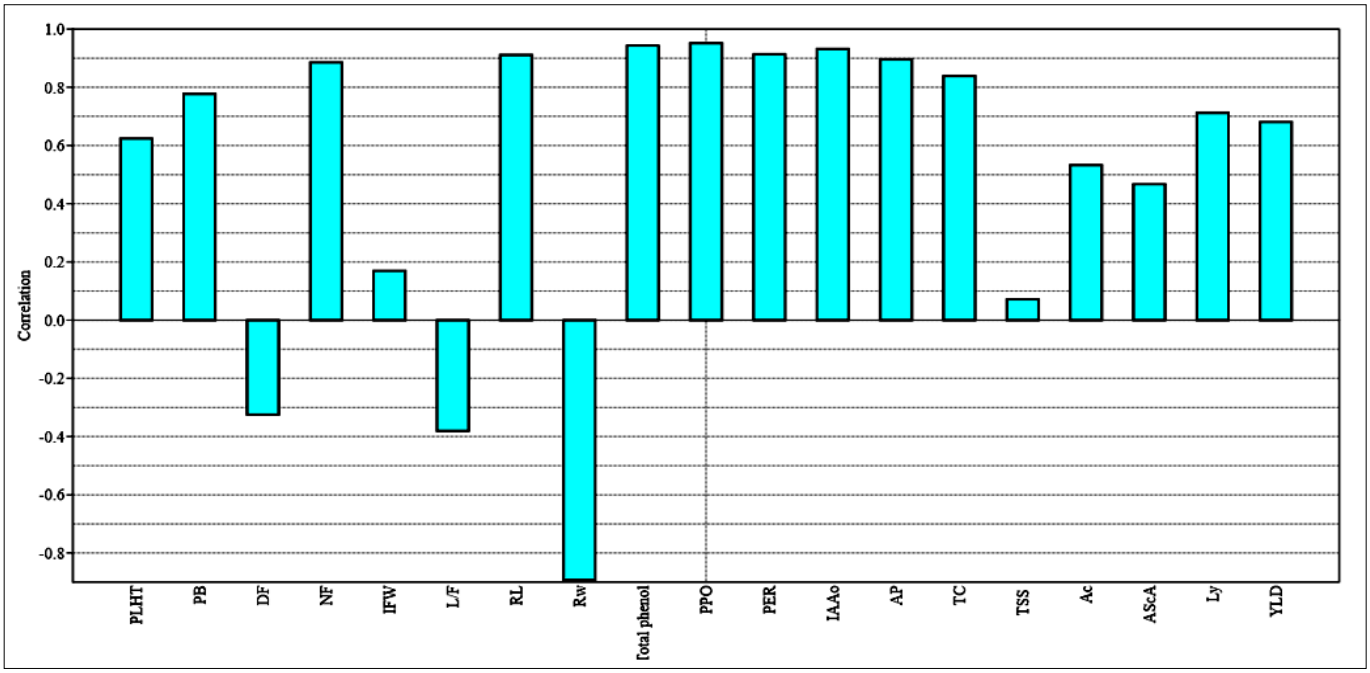

Fig.3. Correlation of traits in PC 1.

From the second component, it was observed that the days to first flowering indicated negative association in both PC_1 and PC_2 (Fig. 4). Thus, the traits derived from three PCs exhibit a significant degree of genetic variation and contribute to unbox the genetic potential among genotypes for crop improvement programmes. The resistant hybrids exhibited earliness compared to susceptible types, indicating strong association of days to first flowering with RKN resistance. Positive contribution indicates significant variation in flowering and fruiting time so it plays important role for selection on the basis of duration (43, 44).

Further, to identify the Genotype associated traits, GT biplot was analysed and represented in graphical form (Fig 5). From Component 1 and 2, it could be observed that the crosses *viz*., Hissar Arun × HN2, Arka Abha × HN2, LE 812 × HN2 and IC 249503 × HN2 exhibited positive association with Total soluble solids, individual fruit weight, Ascorbic acid, yield, lycopene content, primary branches, plant height and number of fruits per plant. These traits can be utilized for screening the crosses. It can also be observed that the parents and Hybrids are in different clusters except HN2 and IIHR 2868. Further the hybrids Arka Abha × HN2 and LE812 × HN2 which are resistant hybrids were closely related (minimum spanning tree method) compared to other crosses and Hissar Arun × HN2 was

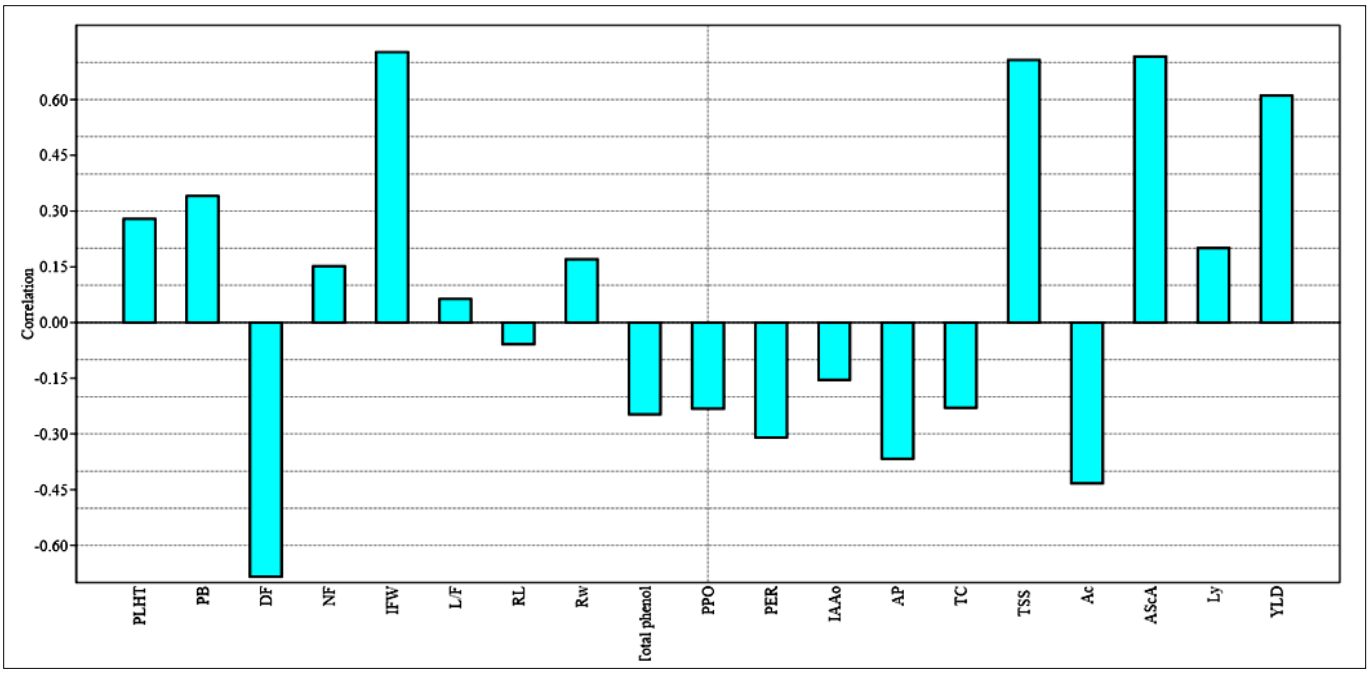

Fig. 4. Correlation of traits in PC 2.

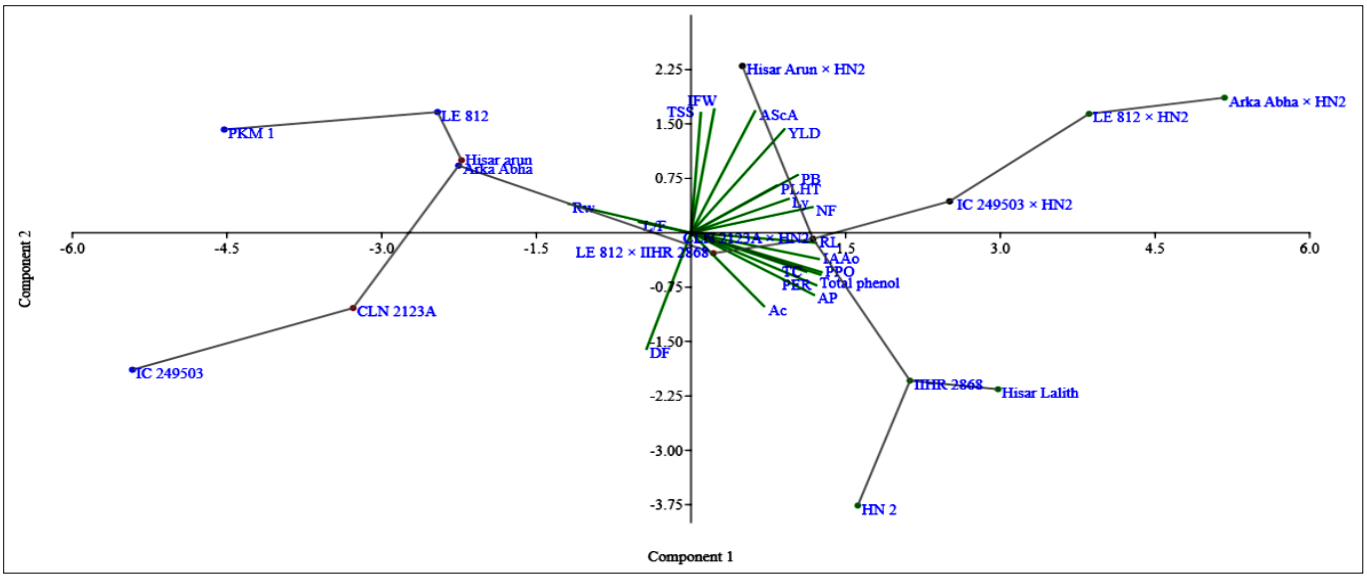

Fig. 5. Genotype trait biplot of tomato.

distinctly different from other crosses. The parents, *viz*., PKM-1, LE812, Hissar Arun and Arka Abha exhibited positive association with root weight and number of locules per fruit and PKM1 was distinct from other parents.

The genotypes IC249503, CLN 2123A were observed to similar genotypes and exhibited strong association based on days to first flowering. IIHR 2868, HN2, Hissar Lalith, LE812 × IIHR2868 and CLN 2123A × HN2 can be screened based on root length, peroxidase activity, Total chlorophyll content, Acid Phosphatase activity, Total phenol, Poly Phenol Oxidase, IAA oxidase and Acidity. The associated characters can used as selection pressure for screening of parents and hybrids.

Ward's cluster analysis

Cluster analysis was used for the identification of different clusters based on the dendrograms of the genotypes evaluated (45). Their effectiveness was well demonstrated (46) and it was indicated that clustering significantly classified the genetic materials which is a positive sign for the breeders to utilize the genotype in crop improvement programmes. In this present study not only parents, hybrids were also utilized to identify the true nature of the hybrids. The practical utility of hierarchical clustering is not only to classify the genotypes, it also classified the traits associated with each other.

In the present investigation, the Wards clustering with Euclidean similarity index (Fig. 6) classified the entire

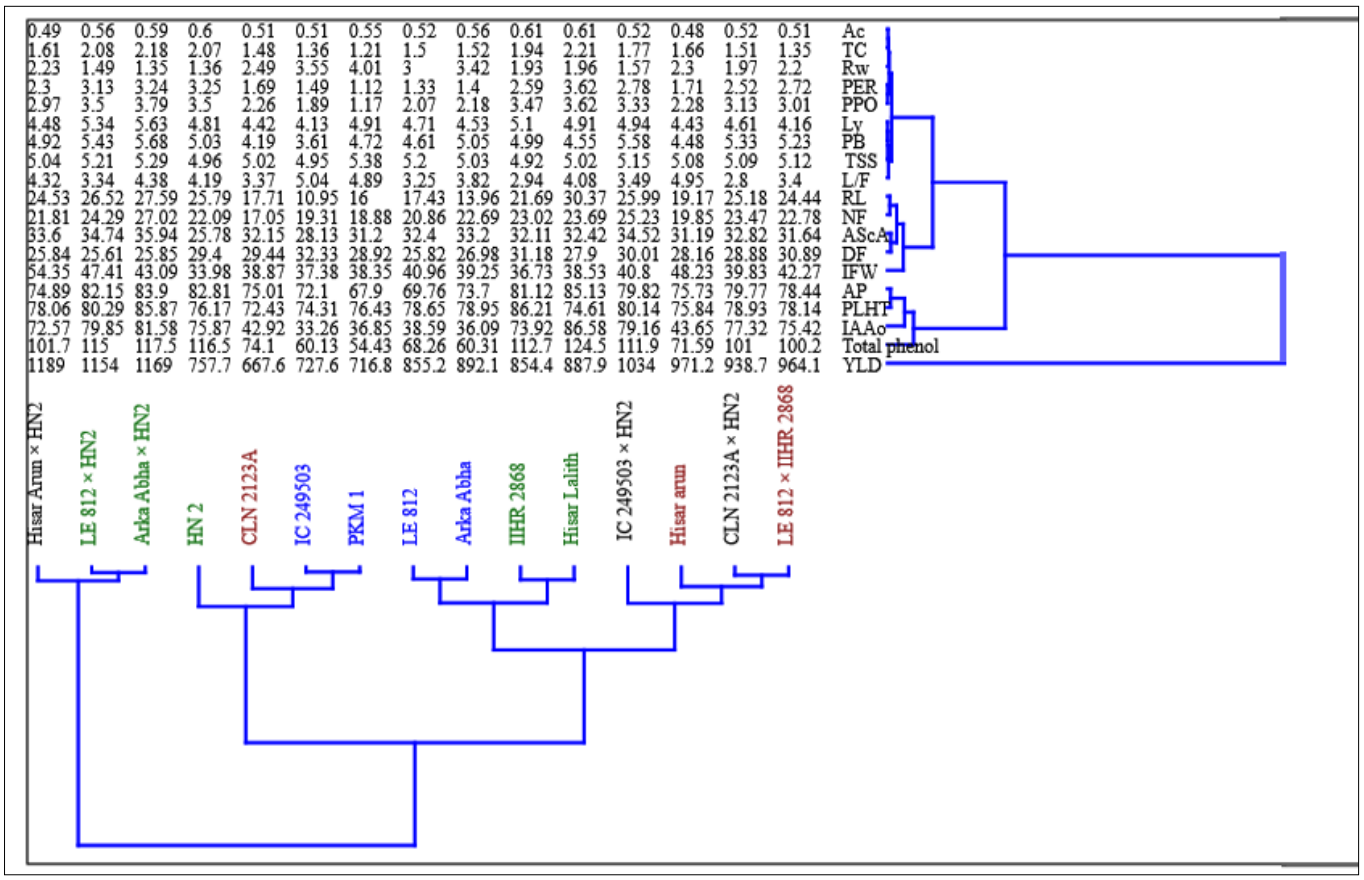

Fig.6. Genotype - Trait analysis by Wards clustering.

dataset into a 3D model *viz*., clustering of genotypes, characters and trait specific genotype. The first model represents the samples of two major sets. The first set included the hybrids *viz*., Hissar Arun×HN2 (susceptible to RKN) and the sub cluster included both LE 812×HN2 and Arka Abha×HN2 indicating the similarity between the hybrids (resistant to RKN). The second cluster had two subsets which had distinguished between parents and hybrids. From the real time data, it can be observed that majority of the physiological parameters of PKM 1 were similar to IC 249503 indicating the genetic potential to grow in similar environmental conditions.

Among the traits under study, two major clusters were obtained *i.e.* yield separated from all other characters indicating the selection pressure on parents and hybrids which was ultimately true for any breeding program.

The second cluster was divided into two subsets. The subset I indicate that the acid phosphatase activity, plant height, total phenol and IAA oxidase where closely related. In indeterminate tomato, the height of the plant may be related to the higher IAA activity while in determinate type of tomato, the IAA activity ceases after the production of IAA oxidase which limits the plant height and can be confirmed with this study. All the parents and hybrids exhibited determinate growth habit indicating the activity of IAA oxidase in controlling the plant height. The subset II was again divided to two clusters, in the first cluster individual fruit weight was distinct. The number of fruits was closely related to the root length and ascorbic acid related to days to first flowering. The second cluster was again divided into two subclusters. In the first subcluster included total chlorophyll, Polyphenol Oxidase (PPO), peroxidase, root weight and acidity. The second subcluster included lycopene, number of primary branches, TSS and locules per fruit.

The inter and intra cluster analysis reveal the trait specific genotype which further dissect the genotype at phenotypic level. In the present investigation, the RKN resistant hybrid Arka Abha×HN2 recorded higher rank for number of fruits (27.02) and in the second dimension, root length (27.59) was closely related to the number of fruits. This exhibits the selection pressure to be applied on this hybrid based on these two characters and not based on root weight. In nematode infested genotypes the incremental root weight was due to formation of root nodules than the normal root structure (Fig 7). The genotype PKM1 exhibited shorter root length (16.00) and lower number of fruits (18.88) may also be interpreted as the genotypes with longer root length may increase the number of fruits.

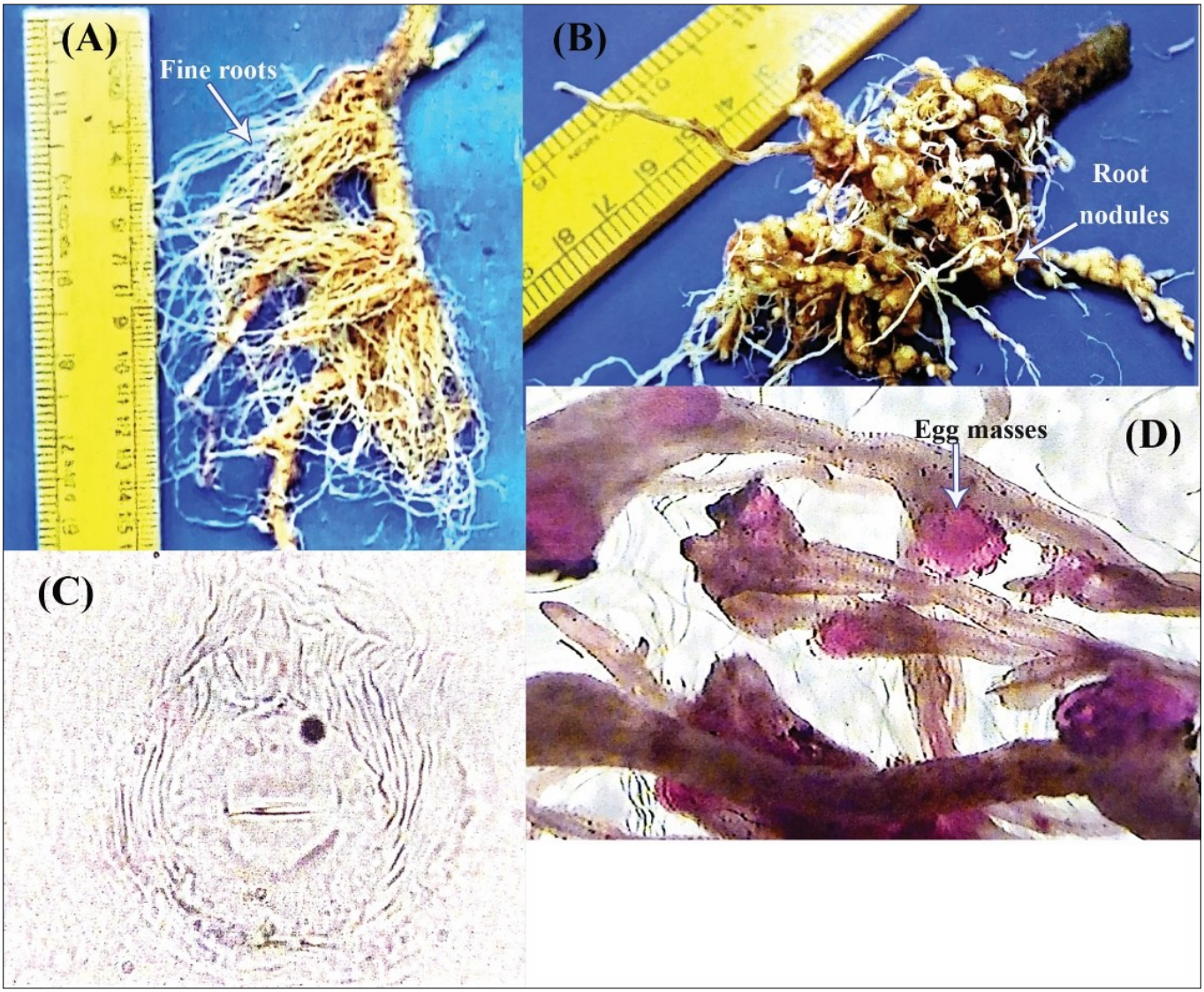

Fig.7.RKN infestation in tomato roots. (**A**).Resistant to RKN, (**B**).Susceptible to RKN, (**C**).Posterior circular fashion and (**D**) Egg masses of RKN.

It was also observed that the yield of both scenario matches with each other *i.e* higher the number of fruits higher the yield and *vice-versa*. This unlocked the genetic potential of genotypes where in at seedling stage, a breeder can pre-identify a better genotype based on the length of the root. Interestingly Arka Abha×HN2 alone registered higher values for Lycopene (5.63), number of primary branches (5.68) and TSS content (5.29) which were under one cluster in the second dimension. It has been reported that negative correlations were observed for number of primary branches, number of fruits and TSS for yield which can also be interpreted as these are associated characters for a genotype, which was evident in study (47). Arka Abha×HN2 and LE 812×HN2 had ranked 1 and 2 respectively with respect to total chlorophyll (TC) and PPO (2.18 & 3.79 and 2.08 & 3.50 respectively) indicate that PPO activity was related to the chloroplast of tomato leaves. The PPO overexpression plants significantly reduced the growth rate and nutritional index of *Helicoverpa armigera* and *Spodoptera exigua in tomato (48).* Hence, these hybrids can be better utilized for low pesticide crop production programs. However, PKM1, LE 812 and Arka Abha registered higher values for TC content than PPO indicating its adaptability to different environmental conditions. Tomato showed increased drought resistance with a lower expression of PPO (49). It can also be observed that both these traits had genotype specific strong association which was illustrated in the two-way cluster.

The hybrid Hissar Arun×HN2 was distinct among the genotype cluster with its distinct trait *i.e.* individual fruit weight in the trait-based cluster. It was also observed that the total phenol and acid phosphatase were similar for the respective genotypes under study indicating that these traits were genotypic specific and the hybrid Arka Abha×HN2 expressed higher values (117.5). Under low phosphorus conditions, plants synthesize acid phosphatases and secrete them into the rhizosphere to scavenge Pi from organophosphate compounds in the rhizosphere (50). In such conditions, the hybrid Arka Abha x HN2 can be recommended for low to medium phosphorous containing soils which is common in Indian soils that affect the productivity of crops (51) and from other perspective, Indian soils are rich in nitrogen and the more total nitrogen, the higher the phosphatase activity in the soil (52). The same hybrid also registered higher values for number of flowers per plant (27.02) which is an important criterion in plant breeding programs.

The score for peroxidase (POD) activity was more pronounced in the hybrids LE 812 x HN2 and Arka Abha x HN2 (79.85 and 81.58). POD eliminates toxic elements in cells, removes excess free radicals in the plant (53) and produces some metabolites needed by cells, thus improving the stress resistance of plants. Hence these hybrids can be well utilized under RKN stress conditions. The cluster analysis in this method is based on Analysis of variance instead of distance. Significantly different genotypes or traits can be distinct and no significant difference in same cluster. It can be observed that two hybrids (LE 812×HN2 and Arka Abha×HN2) were on par and the parent HN2 was significantly different from other two parents. For development of the two hybrids, three parents were used *i.e*. LE 812, Arka Abha and HN2. With regard to earliness in flowering in third dimension, the hybrids expressed higher vigour *i.e*. the hybrid Arka Abha registered 35.94 while the parents Arka Abha (33.20) and HN2 (25.78) and same for the other hybrid LE 812×HN2. Days to first flowering and ascorbic acid were in the same cluster, but their expression differed suggesting that Wards approach efficacy for quantitative features.

Conclusion

Root-knot nematodes (RKN) are polyphagous and infect commercially important crops. Its infestation in tomato at different stages influences the crop growth and yield. From GT-biplot and Ward clustering, it was observed that the cross Hisar Arun x HN2 was closely associated with individual fruit weight and ascorbic acid. The resistant hybrids *viz*., LE812×HN2 and Arka Abha×HN2 were clustered together in Ward clustering similar to the phenotypic data, which indicate the reliability of this statistical model to identify potential varieties and hybrids with highly associated traits.

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Authors' contributions

PRK carried out the experiment. RA carried out the acquisition of data and drafted the article. KK involved in designing of experiment. SM carried out proof reading. MA involved in statistical analysis and MV involved in data interpretation.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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