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Implications of Ripening Inhibiting Mutant Gene (*RIN*) in Different Quantitative and Qualitative Characters of Tomato

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ABSTRACT

A study was conducted comprising of four widely divergent genetic materials of tomato viz. BCT- 111 *rin* possessing specific mutant genes and CLN B, Patharkutchi, BCT-53 are normal lines without possessing specific genes in Central Research Farm, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India during autumn-winter (2018–19 to 2019–20) aimed to evaluate genotype level variation due to *rin* gene in both homozygous and heterozygous condition for different characters. Three hybrids viz. BCT-111 *rin*×CLN B, BCT-111 *rin*×Patharkuchi, BCT-111 *rin*×BCT-53 were developed involving BCT-111 *rin* and normal 3 genotypes. The seven genotypes including the parental lines and hybrids exhibited wide range of variations in different traits namely, plant height (84.59–106.94 cm), number of days to first flowering after transplanting (25.7–41.26), number of flower clusters plant^{-1} (18.21–25.69), number of flowers cluster^{-1} (5.73–8.48), number of fruits plant^{-1} (49.22–76.02), fruit weight (37.89–97.50 g), equatorial diameter (24.71–52.60 mm), polar diameter (26.00–47.81 mm), locule number fruit^{-1} (2.48–3.96), pericarp thickness (4.94–6.84), fruit yield plant^{-1} (2.93–4.07 kg), total soluble solids (3.95–6.21° brix), total sugar content (1.43–3.47%), reducing sugar (1.21%–2.58%), titratable acidity (0.36%–0.64%), ascorbic acid content (25.66–34.76 mg 100 g^{-1} fresh weight), lycopene content (0.38–4.16 mg 100 g^{-1} fresh weight) and β-carotene (0.24–0.60 µg 100 g^{-1} fresh weight). In the genotype possessing the ripening inhibitor rin gene in homozygous condition, fruit did not ripe and lycopene biosynthesis was severely reduced. However, in heterozygous condition, lycopene biosynthesis was almost normal which amply justified the possibility of utilizing the genotype possessing ripening inhibitor rin gene for development of commercial hybrids of tomato.

KEYWORDS: Tomato, heterozygous,homozygous, mutant gene (*rin*), ripening inhibitor

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1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to the nightshade family Solanaceae which is believed to consist of 96 genera and over 2800 species distributed in three subfamilies, Solanoideae (to which *Solanum* belongs), Cestroideae and Solanineae (Knapp et al., 2004). The cultivated tomato is widely grown around the world and constitutes the major input for the agro-based industry. It is also the second most consumed vegetable after potato. Genetic determinants of nutritional quality have long been studied. However, it is only recently that these studies have largely focused on single, or at most, a handful of metabolites, such as carotenoid content in tomato (Liu et al., 2003). Hence, there has been much renewed interest in the possibility of breeding not only higher yielding but also better quality crops. A typical tomato fruit contains intermediate levels of vitamin C, carotenoids, provitaminA, but because of the volume of fresh tomato and tomato products which are consumed, tomatoes make important contribution to the dietary intake (Stommel, 2007, Causse et al., 2007). Tomato as an importantsource of anthocyanins in human food has also been discussed recently (Atanassova et al., 2010).

Today, fruit quality is the major focus of most tomato breeding programmes because of several recent studies highlighting the nutritional importance of lycopene, flavonoids, and chlorogenic acid in the human diet (Devaux et al., 2005, Dixon, 2005, Niggeweg et al., 2006, Rein et al., 2006). The major fruit quality traits of interest to both fresh market and processing tomato industries being fruit size, shape, total soluble solids, lycopene, β-carotene, firmness and flavour while the other important fruit quality characteristics include pH, titrable acidity and vitamin contents (Foolad, 2007). Lycopene makes up approximately 80–90% of the total carotenoids in common cultivars of tomatoes (Shi and Le Maguer, 2000), the pigment that gives tomato its red color. There is considerable interest in the dietary role of lycopene in inhibition of heart disease (Rissanen et al., 2003) and reducing the risk of certain cancers, including prostate cancer (Clinton, 1998, Ansari and Gupta, 2003, Giovannucci, 2002, Wu et al., 2004, Stacewicz-Sapuntzakis and Bowen, 2005) and breast cancer (Sessoet al., 2005). Other carotenoids present in ripe tomato fruits include β-carotene and small amounts of phytoene, phytofluene, d-carotene, z-carotene, neorosporene and lutein (Khachik et al., 2002). β-carotene is the carotenoid recognized as a nutrient in tomato fruit due to its provitamin A activity. Each year, 750 million people suffer from vitamin A deficiency and a single serving of tomato products can supply in excess of 30% of recommended daily allowances. It has been amply justified that total soluble solids content which contain 50% carbohydrates (Helyes et al., 2006) is the most important indicator of the taste of tomato and the fruits containing soluble solids above 4.5 °Brix could be placed in the desirable rank (Clement et al., 2008). Tomato has a very short shelf life due to its perishable nature. Short shelf life coupled with improper processing facilities results in heavy socio-economic loss (Roy and Karmakar, 2019). Delay ripening of fruit or fruit development delays (Wang et al., 2019) plays significant role in shelf life of tomato.

Hence, as a time demanding breeding strategy of improving tomato for enhanced product quality utilizing *ripening inhibitor* (*rin*) mutant gene to gain both domestic as well as international market share as large as possible and availability throughout the year, the following objectives viz. Study on genotype level variation due to *rin* (*ripening inhibitor*) gene in homozygous as well as heterozygous condition compared to normal, non-mutant genotypes for different plant morphological, fruit and fruit quality characters have been framed in the present investigation.

2. MATERIALS AND METHODS

The field experiments were conducted during October November of 2018–19 to 2019–20 at Central Research Farm, Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India. Four widely divergent genetic materials of tomato viz., BCT-111 *rin* possessing *rin* (*ripening inhibitor*) gene and normal lines without possessing specific genes viz., CLN B (heat tolerant and low in carotenoid pigment), Patharkutchi (local adaptable cultivar medium in carotenoid pigment) and BCT-53 (high yielding line with appreciable fruit quality). Following three hybrids were developed through Conventional hybridization method involving BCT-111 *rin* and three normal genotypes viz. BCT-111 *rin*×CLN B, BCT-111 *rin*×Patharkutchi and BCT-111 *rin*×BCT-53. The fruits developed by hybridization were harvested at ripe stage. After keeping those fruits in ambient condition for 4–5 days for completion of physiological maturity of the seeds, the seeds were extracted by fermentation method. The seeds were dried in shade and in sun, both and stored in desiccators for further use. Then evaluation of the hybrids alongwith their parental lines were done following randomized block design with three replications keeping 30 plants replication^{-1} with a spacing of 60×60 cm^2. Standard crop husbandry procedure along with general plant protection measure was followed during the experiment andfive random plants from each genotype were selected for recording the data on different characters. 5 fruits from each of the 5 plants genotype^{-1} were sampled periodically at advanced turning stage and kept in the room temperature condition till the fruits did ripe completely. After taking the morphological

characters of fruit viz., fruit weight (g), equatorial diameter (mm) and polar diameter (mm), the fruits were cut into two halves and pericarp thickness (mm) and locule number was recorded. The cut fruits were used to make replication-wise composite sample to estimate the following fruit quality characters on fresh weight (FW) basis. Total soluble solids (TSS) was determined by hand refractometer and expressed as °Brix.Total sugar content (%) and Reducing sugar (%) were estimated by Anthrone method as per Dubois et al. (1956).Titratable acidity (%)was estimated as per Sadasivam and Manickam (1996) which provides a measure of organic acids in the fruits (expressed as % anhydrous citric acid). Ascorbic acid content (mg 100 g^{-1} fresh weight, FW) was estimated by titration with 2.6-dichlorophenolindophenol sodium salt solution (Anonymous, 1990). For Lycopene content (mg 100 g^{-1} fresh weight, FW) and β-carotene (mg 100 g^{-1} fresh weight, FW)content, homogenized tomato pulp from the composite sample was utilized to determine the amount of lycopene spectrophotometrically (Davies, 1976). Standard Statistical and biometrical analysis was done in the present investigation.

3. RESULTS AND DISCUSSION

The results are presented and discussed here under

3.1. Genotype level variation due to rin gene in both homozygous and heterozygous condition for different characters

3.1.1. Ripening inhibitor (rin) mutant

BCT-111*rin* genotype was semi-determinate in growth habit with normal green leaf. According to Dellapenna et al. (1989) and Giovannoni et al. (1995), the associated macrocalix mutation (*mc*) additionally with *rin* mutation displayed enlarged sepals and loss of inflorescence determinacy, forming shoots at the end of inflorescence, which led to the expression of semi-determinate growth habit. No anthocyanin pigment was visible on stem and petiole. Immature fruits were uniformly light green in colour due to the comparatively low production of chlorophyll pigments. Most characteristic feature of the *rin* genotype was increased size of sepals; green fruit colour at maturity which later turned bright yellow and ripening was retarded for long period. The fruits contained very low lycopene content of 0.38 mg 100 g^{-1} fresh weight as against the average of 3.56 mg 100 g^{-1} fresh weight in three non-mutant normal genotypes (Table 2). It was also recorded earlier that the *rin* mutation decreased lycopene content even in the heterozygous form (Giovannoni et al., 2004, Leseberg et al., 2008).

3.1.2. Chief characteristic features of the genotypes possessing rin gene

The genotype possessing the *rin* mutant gene was semi-determinate in growth habit with normal green foliage. Most characteristic feature of the *rin* genotype was increased size of sepals; green fruit colour at maturity, which later turned bright yellow and fruit, did not turn characteristic red in colour.

3.1.3. Heterozygosity for rin gene

In the present study, 3 hybrids developed through crossing the genotype possessing *rin* gene in homozygous condition (*rin/rin*) and 3 non-mutant normal parental lines in Line×Tester (Mutant×Non-mutant hybrid) mating design were evaluated for plant, fruit and fruit quality characters. Hence, all the hybrids were heterozygous for the *rin* gene (*rin/Rin- -*).

3.1.4. Mean performance

Mean of the plant, fruit, fruit quality and other characters viz., plant height (cm), primary branches $plant^{-1}$, days to first flowering after transplanting, span of flowering, flower clusters $plant^{-1}$, flowers per cluster, fruits $plant^{-1}$, fruit weight (g), equatorial diameter (mm), polar diameter (mm), locule number $fruit^{-1}$, pericarp thickness (mm), seeds per fruit, fruit yield $plant^{-1}$ (g), total soluble solids (°brix), total sugar content (%), reducing sugar (%), titratable acidity (%), ascorbic acid content (mg 100 g^{-1} fresh weight), lycopene content (mg 100 g^{-1} fresh weight) and β-carotene (mg 100 g^{-1} fresh weight), of the 4 parental line and their three mutant×non-mutant hybrids has been presented in Table (1 and 2).

The 7 genotypes under study including the parental lines and hybrids exhibited wide range of variations in ten quantitative traits namely, plant height (84.59–106.94 cm), number of primary branches $plant^{-1}$ (8.26–10.12), number of days to first flowering after transplanting (25.7–41.26), span of flowering (35.61–47.66), number of flower clusters per plant (18.21–25.69), number of flowers $cluster^{-1}$ (5.73–8.48),number of fruits $plant^{-1}$ (49.22–76.02), fruit weight (37.89–97.50 g), equatorial diameter (24.71–52.60 mm), polar diameter (26.00–47.81 mm), locule number per fruit (2.48–3.96), pericarp thickness (4.94–6.84), number of seeds $fruit^{-1}$ (26.32–102.00) fruit yield $plant^{-1}$ (2.93–4.07 g), total soluble solids (3.95–6.21 °brix), total sugar content (1.43–3.47%), reducing sugar (1.21%–2.58%), titratable acidity (0.36–0.64%), ascorbic acid content (25.66-34.76 mg 100 g^{-1} fresh weight), lycopene content (0.38–4.16 mg 100 g^{-1} fresh weight) and β-carotene (0.24–0.60 mg 100 g^{-1} fresh weight), of the 4 parental line and their three mutant×non-mutant hybrids. In the genotype possessing the ripening inhibitor *rin* gene in homozygous condition, fruit did not ripe and lycopene biosynthesis was severely reduced. However, in heterozygous condition, lycopene biosynthesis was almost normal which amply justified the possibility of utilizing the genotype possessing the ripening inhibitor *rin* gene for the development of commercial hybrids of tomato.

Table 1: Mean of different quantitative characters in the parental lines and hybrids

Genotypes	Plant height (cm)	Branches plant^{-1}	Days to first flowering	Span of Flowering	Flower cluster plant^{-1}	Flower cluster^{-1}	Fruits plant^{-1}	Fruit weight (g)	Equatorial diameter (mm)	Polar diameter (mm)
BCT-111 rin	84.59^{b}	8.6^{ab}	36.39^{b}	47.66^{a}	20.82^{ab}	8.48^{a}	49.22^{c}	97.5^{a}	52.6^{a}	47.02^{a}
CLN B	85.47^{b}	10.12^{a}	32^{c}	43.02^{bc}	18.21^{b}	7.42^{a}	76.02^{a}	37.89^{e}	24.71^{d}	26^{c}
Patharkutchi	106.94^{a}	10.04^{ab}	39.25^{ab}	35.61^{d}	24.84^{ab}	7.69^{a}	56.09^{bc}	76.17^{b}	41.5^{c}	35.5^{b}
BCT-53	98.96^{ab}	9.44^{ab}	41.26^{a}	37.07^{d}	25.69^{a}	7.83^{a}	60.67^{b}	82.47^{b}	42.82^{bc}	47.81^{a}
rin×CLN B	98.19^{ab}	8.63^{ab}	25.7^{d}	47.53^{a}	18.62^{b}	5.73^{a}	60.9^{b}	49.73^{d}	43.45^{bc}	43.99^{a}
rin×Patharkutchi	97.59^{ab}	8.26^{b}	29.21^{cd}	39.85^{cd}	18.68^{ab}	5.75^{a}	50.36^{c}	65.26^{c}	48.53^{ab}	46.16^{a}
rin×BCT-53	86.12^{b}	8.73^{ab}	26.33^{d}	45.86^{ab}	19.24^{ab}	5.87^{a}	55.75^{bc}	61.68^{c}	45.23^{bc}	43.28^{a}
SEm±	4.07	0.31	0.68	0.73	1.19	0.48	4.73	1.90	0.99	0.97
CD (p=0.05)	11.62	0.90	1.94	2.08	3.41	1.39	13.49	5.42	2.84	2.79

Table 2: Mean of different fruit quality characters in the parental lines and hybrids

Genotypes	Locule no.	Pericarp thickness (mm)	Seeds fruit^{-1}	Fruit yield plant^{-1} (kg)	TSS (°Brix)	Total sugar (%)	Reducing sugar (%)	Acidity (%)	Lycopene (mg 100 g^{-1})	β-carotene (µg 100 g^{-1})	Ascorbic acid content (mg 100 g^{-1})
BCT-111 rin	2.48^{e}	6.84^{a}	97.02^{a}	4.07^{a}	3.95^{c}	1.61^{bc}	1.46^{bc}	0.36^{c}	0.38^{d}	0.24^{c}	25.66^{b}
CLN B	2.67^{de}	4.94^{d}	72.93^{b}	3.02^{a}	4.4b^{c}	1.43^{c}	1.21^{c}	0.48^{b}	2.66^{c}	0.5^{ab}	27.19^{b}
Patharkutchi	3.62^{ab}	5.76^{bc}	102^{a}	3.66^{a}	6.21^{a}	3.47^{a}	2.58^{a}	0.64^{a}	4.16^{a}	0.57^{a}	27.36^{b}
BCT-53	3.09^{cd}	6.38^{ab}	96.37^{a}	3.78^{a}	4.58^{b}	2.19^{bc}	1.76^{bc}	0.63^{a}	3.87^{ab}	0.6^{a}	29.98^{ab}
rin×CLN B	3.96^{a}	5.62^{cd}	37.32^{c}	2.93^{a}	4.11^{bc}	1.94^{bc}	1.65^{bc}	0.45^{bc}	2.16^{c}	0.37^{bc}	32.02^{ab}
rin×Patharkutchi	3.91^{a}	5.63^{cd}	33.55^{c}	3.39^{a}	4.23^{bc}	2.29^{b}	2.14^{ab}	0.51^{b}	3.66^{ab}	0.46^{ab}	30.06^{ab}
rin×BCT-53	3.34^{bc}	5.69^{bc}	26.32^{c}	3.2^{a}	4.31^{bc}	2.15^{bc}	1.78^{bc}	0.47^{b}	3.43^{b}	0.52^{ab}	34.76^{a}
SEm±	0.08	0.12	2.74	0.20	0.10	0.13	0.12	0.02	0.12	0.02	1.20
CD (p=0.05)	0.23	0.34	7.83	0.58	0.29	0.38	0.34	0.06	0.34	0.08	3.42

4. CONCLUSION

In the genotype possessing the ripening inhibitor *rin* gene in homozygous condition, fruit did not ripe and lycopene biosynthesis was severely reduced. However, in heterozygous condition, lycopene biosynthesis was almost normal which amply justified the possibility of utilizing the genotype possessing the ripening inhibitor *rin* gene for the development of commercial hybrids of tomato. The hybrid BCT-111 *rin*/*rin*×Patharkutchi following by BCT-111 *rin*/*rin*×BCT-53 showed ample promise for further utilization.

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