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Expressivity of two genes controlling functional male sterility in tomato - positional sterile (*ps*) and positional sterile-2 (*ps-2*) during autumn-winter season

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ABSTRACT

The present investigation reports the expressivity of one positional male sterile line (ps) maintained in the Department of Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya and two positional male sterility-2 lines (ps-2) received from Institute of Genetica, Bulgarian Academy of Science, Sofia, Bulgaria in the Eastern Indian condition. Selfed generation of both ps and ps-2 breeding lines under open field condition showed inconsistent functional male sterile expressivity. With a view to 75% plants with stable anther non-dehiscence character along with very low of 3.8 and 5.6 % selfing it appeared that the ps-2 breeding line EC 620175 hold ample promise for utilization in commercial tomato hybrid development in Indian condition. The ps breeding line, BC ps showed comparatively higher selfing percentage. However, selfed population of the "Stable functional sterile plants" of both these lines need to be re-evaluated keeping record of percentage selfing in terms of natural fruit set in each plant to develop an uniform families of "Stable functional sterile plants" possessing least selfing percentage under the present climatic condition.

Key words: Anther non-dehiscence environment, tomato *ps* male sterility, *ps-2* male sterility, pollen trapping

Heterosis is recognized as one of the primary factors that contributed to the success of plant breeding in many crops including tomato. Notwithstanding the autogamous pollination system, the hybrids are presently widely adopted for commercial seed stocks of tomato. The benefit of incorporating male sterility into hybrid breeding programs was recognized not long after the appreciation of the advantages of heterosis and the direction of male sterile genotype in tomato. Now a days male sterility in tomato is recognized as a useful trait in breeding programs that address facilitation and improvement of the process of hybrid seed production.

Genic male sterility is of wide occurrence in tomato, *Solanum lycopersicum*. The majority of male sterile mutants belong to sporogenous (*ms*) male sterile (non-functional pollens due to either pre-meiotic or post-meiotic inconvenience in microsporogenesis related to the development of tapetum) or stamenless (*sl*) male sterile (aberrant stamens and non-viable pollen which is structurally and functionally male sterile) classes, while the frequency of mutations controlling some other genic functional male sterility viz., *ps* (connate petals, normal viable pollens but non-dehiscent anther due to persistent stomium), *ps-2* (normal flower with viable pollens but non-dehiscent anther due to structural alterations in the zone of anther dehiscence), *ex* (viable pollens but long style that protrudes from the anther cone), *cl-2* (failure of the petals to open) and *dl* (growth of epidermal hairs on the anther surface is prevented) is comparatively lower (Kaul, 1987; Sawhney, 1994; Gorman and McCormick, 1997; Atanassova, 1999).

However, inspite of a significant amount of research on the application of genic male sterility in tomato breeding (Rick, 1945; Clayberg, 1965; Lapushner and Frankel, 1967; Philouze, 1974; Durand, 1981; Atanassova and Georgiev, 1986; Stevens and Rick, 1986; Tanskley and Zamir, 1988; Singh and Sawhney, 1998), until present, functional male sterile lines have not been used on a large scale in tomato hybrid seed production. But the application of such type of sterility in practices, although in a limited number of countries, was a potential not to be underestimated in developing approaches that aimed at reducing the time and cost associated with hybrid seed production. Hence, in tomato breeding programme, development of commercially feasible functional male sterility systems is always desired.

The only type of functional male sterility applied in the production of hybrid seeds of tomato is the *ps-2* sterility, particularly in Bulgaria, Czeck Republic and Moldavia mainly because of 100 % *ps-2* progeny by selfing and high yield of hybrid seeds upon use of this line as female parent (Georgiev, 1991; Atanassova, 1999). However, this *ps-2* sterility due to structural alterations in the zone of anther dehiscence causing anther non-dehiscence (Oryol and Zhakova, 1977) is not totally consistent and depends on the environmental conditions (Tronickova, 1962; Philouze, 1978; Atanassova and Gergiev, 1986; Atanassova, 1999).

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Considering the importance of the functional male sterility in basic and applied research, the present investigation was outlined to evaluate the expressivity of the positional sterility (ps) and positional sterility-2 (*ps-2*) characters under open field condition under Eastern Indian condition with a view to their further utilization in hybrid breeding programme.

MATERIALS AND METHODS

The investigation was conducted at Central Research Farm of Bidhan Chandra Krishi Viswavidyalaya, Gayeshpur situated at approximately 23^0 N latitude, 89^0 E longitude and at 9.75 m above mean sea level under the day temperature range of 22.5º to 31.9ºC and night temperature range of 8.4º to 22.4ºC, the average day / night being 27.6º/15.1ºC. Selfed seeds of two *ps-2* functional male sterile breeding lines viz., EC620175 and EC620176 received from the Institute of Genetics, Bulgarian academy of Sciences, Sofia, Bulgaria and one positional sterility line (*ps*),BC *ps* maintained in the Department of Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya constituted the materials for the present study.

Assessment of functional male

The functional male sterile breeding lines were grown under field condition in autumn-winter season (October - March, 2008-09) keeping 40 plants in each of the 3 functional male sterile lines. Functional male sterility in all the selfed populations was assessed by i) spreading cotton wads over the flower buds before anthesis to prevent from pollen contamination and then checking for fruit set (male fertile) or not (male sterile) one week later, ii) examination of anthers under binocular microscope for vertical splitting of anthers inside and subsequent release of pollens in both *ps* and *ps-2* sterility and iii) examining dark brown coloration and dryness of the apex of the anther cone in *ps* sterility.

Percentage of functional male sterile plants

All the flowers in the first 5 flower trusses per plant in each of 3 functional male sterile lines were meticulously examined for expression of the anther non-dehiscent character in the plants. The plants in the 3 functional male sterile lines were regarded as “Stable functional sterile plant” when no fruits in the first 5 flower trusses in the plant was set due to open selfing. The percentage of “Stable functional sterile plant” was determined from the total number of plants in each functional line evaluated. The percentage of selfing was determined as the ratio between the number of flowers in the cyme that set seeded fruits and the total number of flowers per plant.

Characterization of male sterile lines

The stable functional sterile plants were selected at random to record observations on different qualitative characters viz., growth habit, stem pigmentation, leaflet character, flower colour, anther colour, fruit shape and ripe fruit colour; quantitative characters *viz.*, flower buds/cluster, petal length (mm), style length (mm), anther length (mm), plant height (cm), fruit weight (g), equatorial diameter of fruit (cm), polar diameter of fruit (cm), locule number of fruit and pericarp thickness (cm) and different fruit quality characters viz., total soluble solids (^{o} Brix), total sugar content (%), reducing sugar (%), tritrable acidity (%), ascorbic acid content (mg 100^{-1} g flesh), lycopene content (mg 100^{-1} g flesh) and β carotene (mg 100^{-1} g flesh). Five random forced selfed fruits of the “Stable functional sterile plants” of two functional male sterile lines, EC 620175 (*ps-2*) and BC *ps* (*ps*) were used to estimate different fruit quality characters following standard procedures.

RESULTS AND DISCUSSION

Expression of functional male sterility

Positional sterile (*ps*)

Positional sterility controlled by *ps* gene could be characterized by petals showing coalescence of the corolla nearly to their extremity (Fig. 1). The greater lateral growth of the petals causes an overlapping and curling with the adjacent petals. The connate or pseudo-connate form of the petals results in considerable constriction of anthers and tends to hold them in exceedingly close contact with the pistil, particularly at the apex and viable pollen was trapped inside the anther locule. Larson and Paur (1948) much earlier recorded that a stomium that persisted prevented anther dehiscence. Considerable constriction of anther at the apex tended to somewhat protrude the style and stigma beyond the antheridial cone. It was also clear that characteristics and function of *ps* mutant differed from with exerted stigma mutant (*ex*) however, in the *ex* mutant long style of the fully opened flower protrudes from the anther cone and holding the stigma out and away from the concentration of pollen in the central space but in *ps* mutant the petals does not open and are adjoin along with the anther which push the stigma away from the pollen.

Positional sterile-2 (*ps-2*)

Non-dehiscent anthers of the *ps-2* line did not rupture vertically from the tip to liberate the pollens inside it (Fig. 2) contrast to normal rupture of the anthers in the male fertile lines causing release of viable pollens into the central space surrounding the style (Fig. 2).

Fig. 1: Positional sterility (*ps)* functional male sterility (connate petals joined at the apex hindering the liberation of pollens)

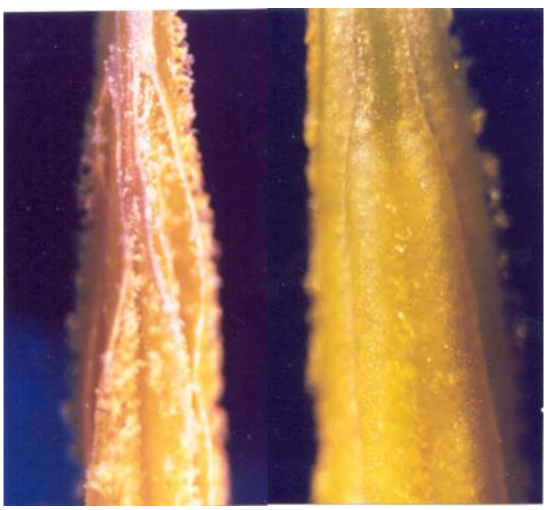

Fig. 2: Positional sterility-2 (*ps-2*) functional male sterility due to non-dehiscent anther (normal vertical anther dehiscence in the left and anther non-dehiscence in the right)

Fig. 3: Comparatively small style of the *ps-2* line EC 620175

The gene *ps-2* resulted from a spontaneous mutant of the Czeck cultivar Vrbicanske nizke and characterized by the presence of fertile pollens and indehiscent anthers (Atanassova, 1991). Comparative studies on anther development in fertile and *ps-2* sterile lines provided evidence that in *ps-2* anthers the zone occupied by endothecial cells with fibrous band thickenings formed on them was three times smaller than in the normal fertile ones and because of such poorer development of the endothecial layer, the power necessary for anther rupture could not be provided resulting indehiscent anthers (Tronickova, 1962, Zhakova, 1977).

Stability of functional male sterility character

Positional sterility-2 (*ps-2*)

It clearly indicated that expressivity of *ps-2* gene largely depended on both the genotype which acquired the *ps-2* gene from the donor spontaneous mutant as well as environmental condition in which it was exposed. The two functional male sterile lines, EC 620175 and EC 620176 acquired the same *ps-2* gene from the original spontaneous mutant. Despite the stability of functional male sterility character in both the breeding lines under the temperate climatic condition of Europe (Bulgaria), the line EC 620176 could not express the stable anther non-dehiscence character under the tropical climatic condition of Eastern India. In the present investigation, EC 620175 showed consistent anther non-dehiscence in 75 % plants through out the flowering span (Table 1). However, in 25 % plants total control on the *ps-2* gene expressivity was not executed resulting open-selfed fruit set in those plants (Table 1). Such environment dependent *ps-2* expressivity in tomato was also recorded earlier in different countries like, France (Atanassova, 1991), Israel (Lapushner and Frankel**,** 1967), Soviet Union (Simonov, 1967) and Bulgaria (Atanassova and Georgiev, 1986). It amply justified elaborate evaluation of the *ps-2* breeding lines to get reliable information on its variability and select stable line for exploring the possibility of its commercial use in hybrid seed production. This functional male sterility proved to be a useful tool in hybrid tomato breeding in Bulgaria, the Czeck Republic, Poland and Moldavia (Pekarkova-Tronickova, 1993; Atanassova, 1999; Atanassova and Georgiev, 2002; Staniaszek *et al*., 2000) and it may become useful in Indian condition also.

Table 1: Stability in functional male sterility in three lines

Functional male sterile line	Plants evaluated	Plants with open fruit set *	Stable functional sterile plant (%)	Range in % selfing
EC 620175 (*ps-2*)	40	10	75.0	3.8 – 5.6
EC 620176 (*ps-2*)	40	40	_0.0	5.8 – 9.5
BC *ps* (*ps*)	40	12	70.0	9.8 – 14.8

*Note: *Anther non dehiscence/ pollen trapping inside the anther was studied upto 5th truss of a plant*

Positional sterility (*ps*)

The breeding line BC *ps* showed ample promise registering 70 % stable functional male sterile plants in the population (Table 1). It was also found earlier that selfing is a disadvantage that also limits the application of *ps* sterility in practice the highest selfing occurred under high temperature condition (Simonov, 1967) which corroborated well to our findings in the present investigation. However, the *ps* mutant was included in a number of breeding programs in different European countries (Nickeson, 1957; Singh *et al*., 1966; Simonov, 1967; Dorossiev, 1976; Staniaszek *et al*., 2000) and this type of sterility may be of practical utility in India also.

Selfed population of the "Stable functional sterile plants" of both EC 620175 (*ps-2* sterility) BC *ps* (*ps* sterility) need to be re-evaluated keeping record of percentage selfing in terms of natural fruit set in each plant of these breeding lines to develop an uniform families of "Stable functional sterile plants" possessing least selfing percentage under the present climatic condition. It need to be studied the discordance, if any, in the anther dehiscence per pollen trapping character also in the population of the "Stable functional sterile plants" of the two populations: i) whether pollen release coincides with stigma receptivity, ii) whether pollen release is late when stigma receptivity has been diminished iii) whether all the anthers have dehisced, iv) whether the pollen release occurred from few anthers v) whether the functional sterility is associated with stigma protrusion and vi) percentage open fruit set in the plants.

Percentage of selfing

We recorded that percentage selfing in terms of natural fruit set was significantly lower in both the *ps-2* breeding lines. In the stable *ps-2* breeding line, EC 620175 selfing percentage ranged between 3.8 and 5.6 percent. In the other *ps-2* breeding line, EC 620176 in which all the plants had open fruit set selfing was also very low ranging between 5.8 and 9.5 per cent (Table 1). This discordance clearly indicated that either anther dehiscence have occurred in few anthers or late when stigma receptivity was diminished. These findings also indicated the possibility of selecting some plants of the EC 620176 possessing lower percentage of selfing. In the *ps* line, BC *ps*, although 70 % plants showed complete functional sterility character selfing in the unstable plants was comparatively high ranging between 9.8 and 14.8 per cent. These findings amply suggested the superiority of *ps-2* functional male sterility in hybrid breeding programme of tomato even in Indian condition. In the present investigation, EC 620175 emerged as the best *ps-2* functional male sterile line.

Maintenance of the functional male sterile lines

Main advantage of utilizing the functional male sterility lay on the maintenance of the male sterile line in homozygous condition by forced selfing. The two comparatively stable functional male sterile lines *viz*., EC 620175 (*ps-2*) and BC *ps* (*ps*) could conveniently be maintained by forced selfing.

However, number of selfed seeds per fruit in all the three lines was much low (Table 2) compared to the fruits of the normal male fertile lines (100 to 160 seeds depending on the genotype).

Table 2: Seed content in the selfed fruits

Genotype	Seeds fruit^{-1}	100 seed weight (g)
EC 620175 (*ps-2*)	25.39 ± 0.52	0.336 ± 0.08
EC 620176 (*ps-2*)	27.46 ± 0.96	0.324 ± 0.07
BC *ps* (*ps*)	36.45 ± 0.88	0.295 ± 0.08

Table 3: Different morphological characters of the breeding lines

Functional male sterile line	Growth habit	Stem pigmentation	Leaf let character	Flower colour	Anther colour	Fruit shape	Ripe fruit colour
EC 620175 (*ps-2*)	Semi-determinate	Pigmented	Potato leaf	Yellow	Yellow	Roundish-flat	Deep red
EC 620176 (*ps-2*)	Semi-determinate	Non-pigmented	Potato leaf	Yellow	Yellow	Blocky-oblong	Orange-red
BC *ps*	Indeterminate	Non-pigmented	Potato leaf	Yellow	Yellow	Oblong	Deep orange-red

Table 4: Characterization of functional male sterile lines

Breeding line	Plant and flower characters				
	Plant height (cm)	Flowers cluster^{-1}	Petal length (mm)	Style length (mm)	Anther length (mm)
EC 620175 (*ps-2*)	90.3 ± 6.3	7.8 ± 0.21	10.3 ± 0.33	5.28 ± 0.21	10.58 ± 0.16
EC 620176 (*ps-2*)	85.7 ± 4.3	6.3 ± 0.16	11.9 ± 0.36	4.92 ± 0.26	10.89 ± 0.13
BC *ps*	156.8 ± 5.7	5.7 ± 0.14	13.4 ± 0.29	8.84 ± 0.11	10.38 ± 0.11
Breeding line	**Characters of the selfed fruits**				
	Fruit weight (g)	**Equatorial diameter (cm)**	**Polar diameter (cm)**	**Locules fruit^{-1}**	**Pericarp thickness (cm)**
EC 620175 (*ps-2*)	70.8 ± 7.8	5.31 ± 0.35	4.15 ± 0.26	3.14 ± 0.06	0.48 ± 0.11
EC 620176 (*ps-2*)	112.6 ± 6.3	6.11 ± 0.36	5.34 ± 0.27	3.62 ± 0.09	0.63 ± 0.12
BC *ps*	64.3 ± 5.3	4.72 ± 0.17	4.63 ± 0.32	2.23 ± 0.11	0.42 ± 0.09
Breeding line	**Quality traits of the selfed fruits**				
	Total soluble solids (^{o} B)	**Total sugar content (%)**	**Titrable acidity (%)**	**Ascorbic acid content (mg 100^{-1} g flesh)**	**Lycopene content (mg 100^{-1} g flesh)**
EC 620175 (*ps-2*)	4.98 ± 0.47	2.73 ± 0.53	0.21 ± 0.44	52.03 ± 0.76	2.32 ± 0.54
EC 620176 (*ps-2*)	4.86 ± 0.66	2.82 ± 0.75	0.26 ± 0.36	48.25 ± 0.64	2.67 ± 0.72
BC *ps*	6.68 ± 0.64	3.94 ± 0.58	0.19 ± 0.38	82.63 ± 0.77	5.01 ± 0.68

Note: Standard error between means of plants in the population

Characterization of the male sterile lines

The stable *ps-2* breeding line EC 620175 was characterized by semi-determinate in growth habit with stem pigmentation and potato leaf character. The fruits are roundish-flat and deep red in colour at ripe stage. The *ps* breeding line BC *ps* was characterized by indeterminate growth habit with non-pigmented stem and potato leaf character. The fruits are oblong and deep orange-red in colour at ripe stage. Petal length, style length and sepal length was the highest in BC *ps*. In the stable *ps-2* breeding line, EC 620175 style length was much lesser than anther length **(Fig.3)** and had fruits of medium weight (64.83g) with medium thick pericarp (0.48 cm) contained 3 locules. All the fruit quality characters including lycopene content (5.01 mg 100^{-1} g fresh) in the *ps* line BC *ps* were significantly better than the *ps-2* lines.

It appeared that the *ps-2* breeding line EC 620175 hold ample promise for utilization in commercial tomato hybrid development in Indian condition.

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