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Introgression of cytoplasmic male sterility (CMS) in tropical carrots (*Daucus carota* subsp. *sativus* Schubl. & Martens)

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Abstract

The petaloid-CMS plants identified in naturally occurring open-pollinated population were used for introgression of male sterility in two varieties (Kashi Arun and Kashi Krishna) and 11 advanced elite materials of tropical carrot through backcrossing at ICAR-IIVR, Jakhini, Varanasi, UP. The tropical varieties and elite materials having diverse morphological backgrounds i.e. root colour (Red, Orange, Yellow, Rainbow, Black & White) and root shape (Danvers, Nantes & Kuroda) were used as recurrent parent in order to recover their background. Thirteen CMS-lines [VRCAR-211, VRCAR-212 & VRCAR-214 (red root); VRCAR-231, VRCAR-234 & VRCAR-241 (orange root); VRCAR-271 & VRCAR-272 (yellow root), VRCAR-291 & VRCAR-292 (rainbow root), VRCAR-251 & VRCAR-252 (black root), and VRCAR-331 (white root)] and their respective maintainers were similar in root colour, root shape and synchronous in flowering/pollination activities which may be used for heterosis breeding for development of nutrient-rich hybrids suitable for tropical/sub-tropical conditions.

Keywords: Tropical carrot, *Daucus carota*, Cytoplasmic male sterility (CMS), Petaloid, Backcross, Hybrid

Introduction

Carrot belongs to family Apiaceae (formerly Umbelliferae), a most nutritious salad vegetable which is cultivated and consumed globally for fleshy edible roots. In 2018, world production of carrot (combined with turnip) was 400 lakh t from 11.31 lakh ha area with China producing 45% of the world total (FAOSTAT 2018). However, during 2018-19 in India, the production, area and productivity was 18.93 lakh t, 1.09 lakh ha and 17.37 t/ha, respectively (Anonymous 2020). The productivity level of carrot in India is very low which is due to predominance of open-pollinated (OP) varieties and lack of high yielding hybrids. Root is basically an enlarged swollen base of the tap root that also includes the hypocotyls. Carrot roots are composed of four distinct sections— peel (periderm), cortex or storage root (phloem, food conducting tissue), cambium and central core (xylem, water conducting tissue). Cultivated carrot has several categories varying in color, shape, size and flavor of roots, and need of vernalization to complete life cycle. Hence, broadly classified into two groups on the basis of (A) presence of root pigments i.e. Carotenoid group (*D. carota* ssp. *sativus* var. *sativus*) and Anthocyanin or purple group (*D. carota* ssp. *sativus* var. *atrorubens*), and (B) need of vernalization i.e. Tropical/Eastern/Oriental carrot (does not require vernalization & annual) and Temperate/Western/European carrot (requires vernalization & biennial) [Singh et al. 2018]. Carrots are distinguished by their distinct root shapes such as Imperator (long and slender root, gradual tapering), Danvers (medium in length, gradual tapering, medium shoulder e.g. Indian red carrot), Triangular (short rooted, more tapering e.g. Indian purple carrot), Chantenay (gradual tapering with blunt end and wider shoulder), Kuroda (Chantenay with medium shoulder), Nantes (almost cylindrical or very less tapered with blunt rounded end), Paris Market (round and short root) and Ox-heart (heart shape). Moreover, the root colour varied from white to black colour (red, orange, yellow, cream, white, purple, black, multi-colour i.e. rainbow); but orange, red and black carrots are most popular and of commercial importance worldwide which are in cultivation among all the climates ranging from temperate to tropics. Mostly the orange carrots are used for table, canning, drying and frozen purposes; and red carrots for table, juice, halwa, salting and pickle making. Furthermore, black carrot (solid purple in some literatures) is very much suitable for healthy salad; preparations of sweets, fresh & fermented juice and purple tea; and pharmaceutical & nutraceutical uses as protective food supplements, healthy food colorants &

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cosmetics (Singh et al. 2021a). Carrots are rich sources of á- and â-carotene (orange carrot), lycopene (red carrot), anthocyanins (black/purple carrot), and lutein/xanthophylls (yellow carrot). The different coloured eye-catching carrots are one of the richest sources of various functional nutrients such as orange carrot for â-carotene (4.5-6.5 mg/100 g FW), red carrot for lycopene (6.0-8.5 mg/100 g FW), yellow carrot for lutein (0.4-0.6 mg/100 g FW) & black carrot for anthocyanins (250-300 mg/100 g FW); and rainbow carrots are blend of all these (2.0-4.0 mg of â-carotene, 4.0-6.0 mg of lycopene, 0.2-0.3 mg of lutein and 15-20 mg of anthocyanins in 100 g FW) [Singh et al. 2021b]. They are also good sources of carbohydrates, minerals and dietary fibre. Black carrot possesses very high anti-oxidative ability i.e. 18 to 23-folds higher than red/orange/yellow carrots, 5-folds higher than beet root, and ranks under five among food items and first among vegetables (Singh et al. 2018, Koley and Singh 2019, Singh et al. 2021a).

Carrot is a cross-pollinated crop (up to 95%), suffering with high inbreeding depression (18-35% for root length, 40-55% for root weight & 62-82% for root yield by Roth 1981) while increasing the homogeneity by selfing of open-pollinated (OP) population/lines/varieties. On the other hand, hybrid vigor/heterosis is quite dramatic between unrelated/diverse lines. Heterosis breeding provides opportunity to harness hybrid vigour for various economic traits as well as to get maximum level of homogeneity/uniformity for traits. The use of F_1 hybrid seed of many vegetable has increased manifold during the past 3-5 decades in India and many countries. Heterosis is of direct interest for development and commercialization of F_1 hybrids in vegetable crops, including carrot which is being triggered by use of various types of CMS.

The first report of heterosis in carrot upon crossing of two inbred lines was made by Poole (1937). Moreover, Frimmel and Lauche (1938) were the first to recommend the breeding of F_1 carrot varieties; but the lack of a system of producing hybrid seed in large quantities prevented the introduction of this method. The male sterility in carrot was discovered and analyzed by Thompson (1962) and Hanschke & Gabelman (1963) which broadly promotes hybrid breeding. The heterosis breeding in carrot is based on three systems of CMS with different genetic backgrounds i.e. (i) brown anther type with Sa-cytoplasm, (ii) petaloid type with Sp-cytoplasm and (iii) gummifer type with Sgu-cytoplasm. Amongst them, Petaloid-CMS system is most stable across a wide range of environmental conditions and is being commercially used for carrot heterosis breeding globally. Petaloid-CMS is manifested by the replacement of either stamen with petals or both stamens & petals with green bract like structures (Kitagawa et al. 1994). Hybrid breeding is considered a promising mechanism to increase root yield and uniformity (Duan et al. 1996, Suh et al. 1999, Simon and Strandberg 1998, Jagosz 2012, Singh 2017, Turner et al. 2018), carotenoid content (Santos and Simon 2006), and resistance to Alternaria leaf blight (Simon and Strandberg 1998). However, the development of CMS lines & heterotic hybrids of carrot in India remains very limited. Verma et al. (2002) and Gupta et al. (2006) have reported heterosis in temperate carrot. Further, Kalia et al. (2019) had transferred CMS system in red, orange and purple coloured Asiatic carrots. Keeping in view of the importance of nutrient-rich coloured carrots and CMS system in heterosis breeding, introgression of male sterility trait in tropical carrots initiated following backcross method at ICAR-IIVR, Varanasi, UP in the various backgrounds of economic importance i.e. root colour (Red, Orange, Yellow, Rainbow, Black & White) and root shape (Danvers, Nantes & Kuroda).

Materials and Methods

The introgression of petaloid-CMS trait in various tropical carrots was carried out following backcross method at ICAR-IIVR, Jakhini, Varanasi, UP from 2013-14 and onwards. The economically important morphological traits of 13 introgressed CMS lines (BC_3-BC_6) along with their maintainers (fertile lines i.e. B-lines) were evaluated in the present study. The source of petaloid-CMS for all pigmented CMS lines [VRCAR-211, VRCAR-212 & VRCAR-214 (red root); VRCAR-231, VRCAR-234 & VRCAR-241 (orange root); VRCAR-271 & VRCAR-272 (yellow root), VRCAR-291 & VRCAR-292 (rainbow root), VRCAR-251 & VRCAR-252 (black root)] was a naturally occurring plant in an open-pollinated population having orangish-red coloured roots; moreover, petaloid-CMS source for VRCAR-331 (white carrot) was identified in its maintainer population having creamish-white coloured roots. The maintainer of VRCAR-331 was originally isolated from a population having cream coloured roots. The identified CMS plants were used as female parent in first cross with two varieties (Kashi Arun and Kashi Krishna) and 11 advanced elite materials as male or recurrent parent. Thereafter, 3-6 generations of backcrosses were made with recurrent parent in order to recover the respective agronomic background. In each cross, 50-100 primary/secondary umbels were pollinated. In every generation, an umbel from 5-10 plants of recipient population was bagged just before anthesis in order to ensure complete male sterility as indicated by no seed set. The stable CMS lines (A-line) were maintained by crossing with their respective B-line; and the maintainers were maintained by selfing & sib-matting process in alternate years to avoid inbreeding depression. The crops were grown in open field for root development (October to mid-January) and generation was advanced through by backcrossing (mid-January to mid-June) at the Research Farm, ICAR-IIVR, Varanasi, UP. The experimental farm is located at 25°10'55'' N latitude, 82°52'36'' E longitude and with an altitude of 85 m above the mean sea level, and receives an annual rainfall of 1050-1100 mm. The soil of the Farm was of silt-loam in texture, having pH of 7.3 and electrical conductivity of 0.28 dSm^{-1}. The crops for root as well as seed development were fed with fertilizers such as $N:P_2O_5:K_2O$ @ 80:50:40 kg/ha and 80:60:60 kg/ha, respectively which was supplied in the form of urea, single super phosphate (SSP) and muriate of potash (MOP). Half dose of N, and full dose of P_2O_5 and K_2O were applied as basal at final plot dressing; and rest half dose of N was top-dressed at 60 & 30 days after seed sowing and steckling transplanting, respectively. The other agronomical practices of experimental fields were carried out almost unvaryingly as mentioned by Singh and Karmakar (2015) for better crop growth, root development and seed development.

Carrot seeds, botanically schizocarp/mericarp, were sown at 1.0-1.5 cm interval in double row of 7-8 cm apart and 25-28 cm wide ridge with the spacing of 65 cm between each pair of 6.00 m long ridges. To keep the plant to plant spacing about 4-5 cm apart, manual thinning was done after about 20 days after seed germination for proper root development. Moreover, the stecklings were prepared by cutting plants leaving 5-6 cm long of root portion and 2-3 cm of leaf petiole; and transplanted at the spacing of 65×50 cm. The stecklings of maintainer lines were transplanted in four rows. Moreover, for backcrossing, the stecklings were transplanted in 3:1 pattern i.e. three rows of CMS lines and one row of maintainer. All four rows were covered with a 24×24 mesh-sized flexible UV stabilized nylon-net cage at the time of umbel formation to avoid chance of any cross-contamination. At the flowering stage, backcrossing was done by transfer of pollen grains of maintainer line to respective CMS line, and selfing or sib-matting in maintainer lines with the help of muslin-puffs manually. The seeds of primary and secondary umbels were harvested at proper time, threshed, cleaned, packed hermetically and stored safely for further sowing. The pooled data for vegetative, root and flowering traits were analysed statistically using Microsoft Excel Software. The values of quantitative traits were presented in bar chart and compared with standard error bars with $p<0.05$ to identify homogeneity of data among CMS lines/maintainers.

Results and Discussion

Male sterility is the inability of the plant to produce fertile pollen grains but maintain female fertility which provide one of the most efficient and direct controlled pollination for hybrid seed production on large scale. Therefore, the plant breeders had begun to pay more attention to the genetic sources of male sterility, transfer it in to elite background, and use of them in heterosis breeding and commercial hybrid seed production. However, CMS is an extra-nuclear maternally transmitted trait due to the structural rearrangements of mitochondrial DNA which entails a dysfunction of respiratory metabolism of male gamete production while the female fertility remains unaffected.

Backcross breeding is the most commonly used method for incorporating male sterility gene into desired elite background. With each successive backcross, the expected recovery of the recurrent parent increases (i.e. 50% in F_1, 75% in BC_1F_1 to 99.22% in BC_6F_1) and contribution from donor line decreases (50% in F_1, 25% in BC_1F_1 to 0.78% in BC_6F_1) except male sterility trait. The introgression of petaloid-CMS trait of carrot in 13 varieties & breeding lines having diverse morphological backgrounds i.e. root colour (Red, Orange, Yellow, Rainbow, Black & White) and root shape (Danvers, Nantes & Kuroda) were carried out, and evaluated in backcross stage of BC_3-BC_6 along with their maintainers for traits of economic importance such as exterior root colour, core colour, root shape, major pigment, leaf colour, petiole colour, gross plant weight, root length, root weight, shoulder diameter, days to 50% flowering and plant height at flowering (Table 1, Figure 1-6).

The qualitative traits such as root & core colour, root shape, leaf colour and petiole colour of CMS lines (Table

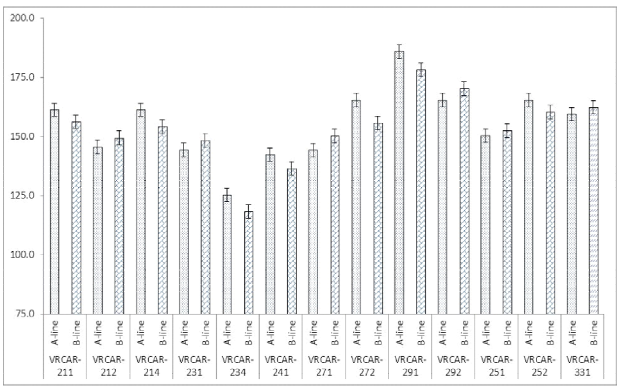

Figure 1: Gross plant weight (g) of 13 CMS lines and their maintainers of coloured tropical carrots

Table 1: Qualitative traits of CMS lines of different coloured tropical carrots

CMS line	Backcross stage	Root colour (Exterior)	Core colour	Root shape	Major pigment	Leaf colour	Petiole colour
VRCAR-211	$BC_6 F_1$	Red	Red	Danvers	Lycopene	Green	Light green
VRCAR-212	$BC_6 F_1$	Red	Red	Danvers	Lycopene	Green	Light green
VRCAR-214	$BC_6 F_1$	Red	Red	Danvers	Lycopene	Green	Light green
VRCAR-231	$BC_6 F_1$	Light orange	Light orange	Danvers	β-carotene	Green	Light green
VRCAR-234	$BC_4 F_1$	Orange	Orange	Nantes	β-carotene	Green	Light green
VRCAR-241	$BC_4 F_1$	Orange	Orange	Kuroda	β-carotene	Green	Light green
VRCAR-271	$BC_6 F_1$	Yellow	Yellow	Danvers	Lutein	Light green	Light green
VRCAR-272	$BC_6 F_1$	Yellow	Yellow	Danvers	Lutein	Light green	Light green
VRCAR-291*	$BC_6 F_1$	Purple	Orangish-red	Danvers	#	Green	Purplish-green
VRCAR-292*	$BC_6 F_1$	Purple	Orangish-red	Danvers	#	Green	Purplish-green
VRCAR-251	$BC_6 F_1$	Black^	Black	Danvers	Anthocyanins	Purplish-green	Purple
VRCAR-252	$BC_6 F_1$	Black^	Black	Danvers	Anthocyanins	Purplish-green	Purple
VRCAR-331	$BC_3 F_1$	White	White	Danvers	Colourless	Green	Light green

*: Multi-coloured root i.e. rainbow carrot; ^: Also known as Solid purple**;**
#: Anthocyanins + Lycopene + â-carotene + Lutein;

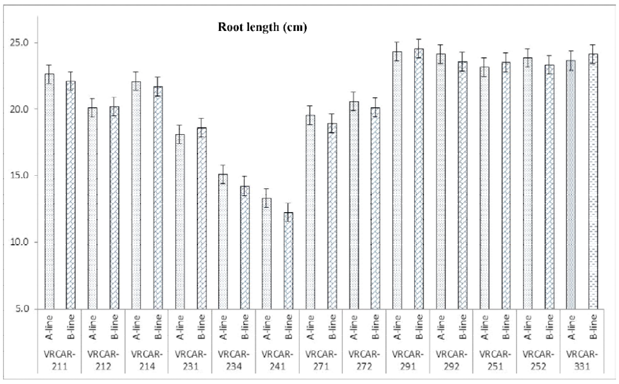

Figure 2: Root length (cm) of 13 CMS lines and their maintainers of coloured tropical carrots

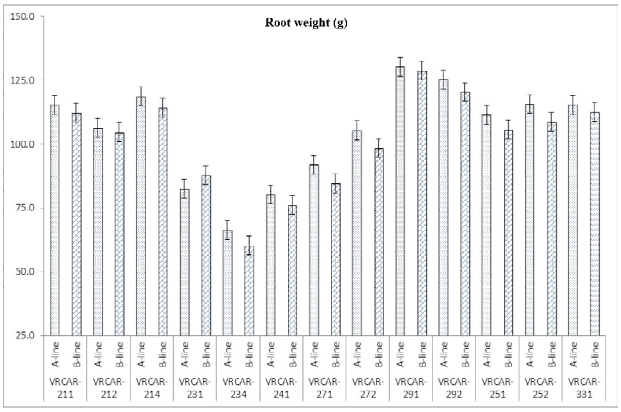

Figure 3: Root weight (g) of 13 CMS lines and their maintainers of coloured tropical carrots

1) were similar to their maintainer lines in BC_3 stage for red, yellow, orange and white carrots; moreover, the desired root colour uniformity of A- & B-lines for black and rainbow carrots was achieved in BC_5 stage. Since the colour of root interior of F_1 cross between Red/ Orange × Black carrot is yellowish-cream and the

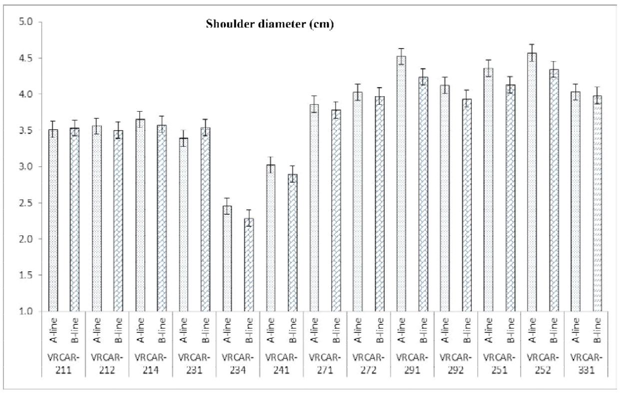

Figure 4: Shoulder diameter (cm) of 13 CMS lines and their maintainers of coloured tropical carrots

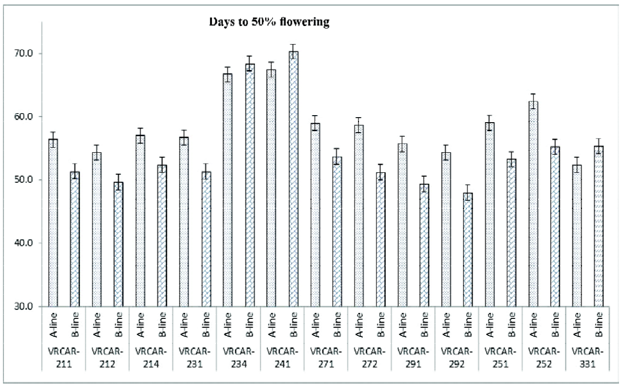

Figure 5: Days to 50% flowering of 13 CMS lines and their maintainers of coloured tropical carrots

anthocyanins pigmentation pattern of in exterior and interior parts of root in black carrot is centripetal; hence taking two more generations of backcrosses for getting uniform root colour. The gross plant weight ranged from 125.3-185.9 g (x=155.1 g) for CMS lines which was statistically in same range i.e. 118.4-178.2 g (x=153.3 g) for their maintainers (Figure 1), except for two CMS

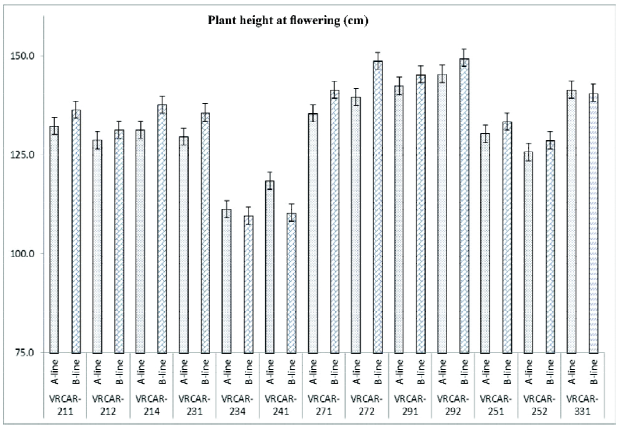

Figure 6: Plant height at flowering (cm) of 13 CMS lines and their maintainers of coloured tropical carrots

lines, VRCAR-272 and VRCAR-292. Generally, gross plant weight was higher for CMS lines as compared to respective maintainer line which might be due to absence of inbreeding depression in CMS lines.

Moreover, root length for CMS lines and corresponding maintainers was statistically at par for all the genotypes which varied from 13.3-24.4 cm (x=20.8 cm) and 12.2-24.6 cm (x=20.6 cm), and was minimum (12-15 cm) for Nantes and Kuroda type genotypes (VRCAR-234 and VRCAR-241; Figure 2). Overall, like gross plant weight, root length was generally higher in CMS lines as compared to their maintainer. Similar to root length, average root weight of 13 CMS lines and its maintainers was statistically at same level which ranged from 66.4-130.3 g (x=105.0 g) and 60.4-128.4 g (x=101.1 g), respectively and was minimum (60-80 g) for Nantes and Kuroda type genotypes (VRCAR-234 and VRCAR-241; Figure 3). Overall, like gross plant weight, root weight was generally higher in CMS lines as compared to their maintainer. Furthermore, the shoulder diameter ranged from 2.5-4.6 cm (x=3.8 cm) for CMS lines which was at par i.e. 2.3-4.3 cm (x=3.7 cm) with their maintainers (Figure 4), except for one CMS line, VRCAR-291. It was minimum (2.3-3.5 cm) for Nantes and Kuroda type genotypes (VRCAR-234 and VRCAR-241; Figure 4) followed by red carrots (3.5-3.7 cm), yellow carrot (3.8-4.0 cm), rainbow carrot (3.9-4.5 cm) and black carrot (4.1-4.6 cm).

Days to 50% flowering was significantly longer (4-7 days) in newly developed CMS lines as compared to its maintainers, except two genotypes VRCAR-234 and VRCAR-241 because of early generation of backcrossing (BC_4F_1), and was ranged between 52-67 days (x=58 days) and 48-70 days (x=55 days), respectively (Figure 5). Although the maintainers flower 4-7 day early to its CMS line yet the time of receptivity of stigma of CMS line and anther dehiscence of maintainer line is almost same i.e. synchronous in flowering/pollination activities which is a prerequisite for maintenance of CMS line (A-line). Nantes and Kuroda type genotypes (VRCAR-234 and VRCAR-241) took longer duration of about 66-70 days for flowering as compared to the other 11 genotypes of tropical carrot as the maintainer of both lines were developed by tropicalizing temperate-specific traits. Unlike to days to flowering, the plant height at flowering for 13 CMS lines and its maintainers (Figure 6) was varied from 111.3-145.4 cm (x=131.6 cm) and 109.7-149.4 cm (x=134.5 cm), respectively which was generally higher for maintainer lines with few exceptions. Plant height was longer for genotypes belonging to rainbow (142-150 cm) followed by white (141-142 cm), yellow (135-149 cm), red (129-138 cm), black (126-134 cm) and shorter in Nantes and Kuroda type genotypes (110-119 cm).

Carrot hybrid development programme is based on three-line system (A-, B- & C-line), the maintenance of CMS lines is very crucial. An ideal CMS line should flower at same time or few days earlier than the maintainer line to get proper pollination and maximum seed set in the CMS line, and also A-line should be slightly shorter in height for abundance availability of pollen grains. The results indicated that all 13 CMS-lines and its respective maintainer were ideally synchronous in flowering/ pollination activities. Therefore, it may be concluded that these newly developed CMS lines of red, orange, yellow, rainbow, black & white coloured tropical carrots may be used intensively for development of nutrient-rich hybrids for tropical/sub-tropical conditions so that breakthrough may be achieved in productivity and colour/pigment intensity of carrot.

सारांश

गाजर की प्राकृतिक रूप से उपलब्ध मुक्त परागित समूह से पेटलोयड–कोशिकाद्रव्यी नर बन्ध्यता का अनुक्रमण दो नर बन्ध्य किस्मों (काशी अरूण तथा काशी कृष्णा) तथा आई.एल. उच्चीकृत सर्वोत्कृष्ट उष्णकटिबंधीय प्रभेदों जो प्रतीप संकरण से विकसित थी, का मूल्यांकन भा.कृ.अनु.प.–भारतीय सब्जी अनुसंधान संस्थान, वाराणसी (उ.प्र.) में किया गया। उष्णकटिबंधीय किस्मों एवं सर्वोत्तम सामग्री जिनमें विविध आकारकीय पृष्ठभूमि वाले गुणों जैसे–जड़ का रंग (लाल, नारंगी, पीला, इंद्रधनुशी, काला व सफेद) तथा जड़ आकार (डेन्वर्स, नैन्टस व कुरोडा) का उपयोग आवर्ती पितृ के रूप में उनके पृष्ठभूमि को प्राप्त करने के लिये किया गया। कुल 13 कोशिकाद्रव्यी नर बन्ध्य लाइनों (वी.आर.सी.ए.आर.–211, वी.आर.सी.ए.आर.–12 व वी.आर.सी.ए.आर.–214 (लाल जड़); वी.आर.सी.ए.आर.–231, वी.आर.सी.ए.आर.–234 व वी.आर.सी.ए.आर.–241 (नारंगी जड़); वी.आर.सी.ए.आर.–271 व वी.आर.सी.ए.आर.–272 (पीला जड़), वी.आर.सी.ए.आर.–291 व वी.आर.सी.ए.आर.–292 (इन्द्रधनुषी जड़),

वी.आर.सी.ए.आर.–251 व वी.आर.सी.ए.आर.–282 (काला जड़) तथा वी.आर.सी.ए.आर.–331 (सफेद जड़) एवं उनके सम्बन्धित अनुरक्षक भी जड़ रंग, जड़ आकार व एक समय पर पुष्पन/परागण प्रक्रिया भी एक समान थी जिसका उपयोग पोषक में धनी उष्णकटिबंधीय/उपोष्णकटिबंधीय उपयुक्त संकरों का विकास ओज प्रजनन के माध्यम से किया जा सकता है।

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