Morphological Characterization of Anther Derived Plants in Minipaprika
(Capsicum annuum L.)

Binod Prasad Luitel, Prakash Babu Adhikari, Surendra Lal Shrestha, and Won Hee Kang*

Department of Horticulture, Kangwon National University, Chuncheon 200-701, Korea

Abstract: This study was done to characterize the anther derived regenerants (R1) including haploids and spontaneous diploids of minipaprika (cv. ‘Vine sweet-red’ ‘Vine sweet-yellow’ and ‘Vine sweet-orange’) in glasshouse. Eleven haploids (three, seven and one from red, yellow and orange, respectively) and sixteen spontaneous diploids (five, nine and two from red, yellow and orange, respectively) were grown in plastic pot with three (red, yellow and orange) anther donor (R0) F1 minipaprika varieties. Regenerants were characterized for their plant and fruit characters as well as their fruit color and shape. The homozygosity of spontaneous diploid plants of each population was assessed using simple-sequence repeat (SSR) marker analysis. Haploid plants were characterized by reduced plant height, small leaves, short petiole and internode and small flower bud and all haploids showed the sterility and vice-versa in spontaneous diploid lines. The fruit biometric traits exhibited greater variation within the spontaneous diploid plants and average value of quantitative traits is lower than standard varieties. MR-4 gave the highest yield (150.5 g) per plant followed by MY-6 (140.0 g) and MY-8 (130.5 g) and the lowest in MY-5 (31.5 g). Morphological marker such as fruit color further determined the microspore origin of androgenic diploids obtained in anther culture of ‘Vine sweet-red’. Of the fifteen spontaneous diploid plants, fourteen plants were identified as doubled haploids using microsatellite markers (SSR), and these homozygous lines are recommended to use in minipaprika breeding program.

Keywords: Haploids, Spontaneous diploids, Homozygosity, Simple-sequence repeat, Yield

INTRODUCTION

In vitro anther culture is the recent breeding method to obtain the haploid and doubled haploid (DH) plants in pepper (Gemesne et al., 2009; Nowa czyk et al., 2009; Pauk et al., 2010; Irikova et al., 2011). The morphological evaluation of doubled haploid lines is important to differentiate the particular lines (Olszewska et al., 2011). The morphological characterization of anther culture derived plants is useful for pepper breeding program (Shrestha et al., 2011). The uniform fruit size with blocky, lamuyo shape, smooth and shiny surface, uniform color, thick and firm pericarp, and good storability are the requirements for developing the new variety in minipaprika. In addition, earliness, fruit quality including flavor and pungency, pedicel length, and multiple fruitedness are important horticultural characters for the pepper breeding (Greenleaf, 1986).

The in vitro culture of anther follows two modes of androgenesis that lead to the development of the haploids either directly via pollen embryogenesis or indirectly via callus formation (Bajaj, 1983). Since the callus-derived plants show the genetic variation, pepper breeders prefer the plants develop from an unreduced microspore origin. The homozygous dihaploid plantlets derived from microspores have direct value to breeding program and doubled haploids (DHs) have been widely used to develop the varieties in peppers (Pauk et al., 2010; Dolcet-Sanjuan, 2005) and asparagus (Falavignaet al., 1999; Corriolset al., 1990). The ‘spontaneous duplication’ of chromosome during the anther culture results dihaploids in pepper (Sibi et al., 1979; Dumas De Vaulx et al., 1981; Vagera and Havranek, 1985; Vagera, 1990). The pollen-derived homozygous diploid plants show the normal meiotic segregation and they do not loss the desirable characters by segregation. Therefore,
it is important to determine the origin of diploid regenerants (Munyon et al., 1989; Olsewska et al., 2011) obtained from anther culture. The fruit characters are also very useful for the confirmation on microspore origins of pepper androgenic plants, determined by single particularly recessive genes, e.g. fruit color and shape (Smith, 1950; Pochard, 1977). The origin of anther culture derived regenerants and their homo- and heterozygosity were determined using enzyme markers (Munyon et al., 1989; Dolcet-Sanjuan et al., 1997; Olsewska et al., 2011). Likewise, the confirmation of genetic homogeneity within the plants of particular DH lines and the polymorphism between the different lines is also possible through PCR based DNA markers (Gyulai et al., 2000; Gemesne et al., 2001). The objectives of this study were to analyze the plant and fruit morphological characters of anther derived regenerants (R1) and to confirm the homozygosity of spontaneous dihaploid plants obtained from the anther culture of minipaprika using SSR markers.

MATERIALS AND METHODS

Growing regenerants (R1) in glasshouse
The number of plants regenerated from anther culture of minipaprika cv. ‘Vine sweet-red’, ‘Vine sweet-yellow’ and ‘Vine sweet-orange’ were 9, 33 and 3, respectively (Luitel, 2012). After the ploidy analysis of regenerated plants, haploids and spontaneously diploid plants were separated from each group and grown at pot (30 cm × 27 cm × 17 cm) in glasshouse, at Kangwon National University (KNU) in Feb. 2012. The haploid plants survived during the acclimatization were 3 (75%), 7 (50.0%) and 1 (100%) in red, yellow, and orange forms respectively, whereas 5 (100%) 9(47.3%), and 2 (100%) diploid plants were survived in red, yellow and orange forms of minipaprika, respectively (Luitel, 2012). The F1 seedlings of red, yellow and orange minipaprika (or anther donor plants, R0) were transplanted in pots for the comparison. The regenerants were named as minipaprika (M) series on the basis of fruit color of their anther donor genotypes. The plants were watered daily and fertilizer was supplied as poly-feed®, soluble nitrogen, phosphorous and potash (NPK) (11-8-34+2 Mg) as per the plants requirement.

Morphological characterization of regenerants
Haploids and spontaneous diploids were characterized for their plant height (cm), leaf size (leaf length and width), petiole length, internode length, flower bud size (length and width), nodal color, leaf color and leaf shape after 50 days of transplanting in the pots, and compared with anther donor plants of anther culture. Vernier caliper was used to measure the flower bud size. In diploid plants of each minipaprika, fruit per plant, fruit weight, fruit yield per plant, fruit length and width, fruit shape index (length/width ratio), fruit volume, pericarp thickness, length of the fruit stalk, fruit stalk weight, fruit set, maturity, fruit size, stalk cavity, fruit lobe number, immature and mature fruit color, fruit shape, fruit shape at apex, fruit surface, and number of seed per fruit were observed. Fruit yield per plant was calculated by counting and weighing all the fruits per plant. Plant and fruit characteristics were evaluated according to the principles given in ‘Descriptors for Capsicum (Capsicum spp.)’ (IPGRI, 1995), and general descriptors developed in minipaprika.

Identification of homozygosity using SSR markers
Total genomic DNA was extracted from fresh young leaf tissue from anther derived spontaneously diploids (5, 9 and 1 lines from red, yellow, and orange, respectively) and anther donor (parent) plants using a simplified 2% CTAB method (Doyle and Doyle, 1990).

Seven microsatellite primers; 1.(ACTG)₄, 2.(GACA)₄, 3.(GATA)₄, 4.(GACAC)₄, 5.‘GACAGATAGACAGACA’-3’, 6.‘GACAGATAGACAGATA’-3’ and 7. ‘GACAGACAGATACATA’-3’ were obtained from Macrogen Co. (Seoul, Korea). These primers were initially developed for tomato (Gupta et al., 1994) and later, primers 1 and 2 were used for Capsicum (Gyulai et al., 2000) to identify the doubled haploids. The annealing temperature was 45°C (primers 1 and 2), 43°C (primer 3), 55°C (primer 4), 48°C (primer 5)