Original Research Article

Korean J. Plant Res. 23(6) : 503～512 (2010)

Agronomic Variation in Anther Derived Plants of Sweet Pepper (*Capsicum annuum* L.) Genotypes

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Abstract - Anther derived double haploids (DHs) from sweet pepper genotypes ('Special', 'Derby', 'Bossanova', 'Fiesta', 'Debora' and 'Minipaprika') were used to study the agronomic variation in 2006. Ninety-nine successful DHs regenerants (32 from 'Special', 25 from 'Derby', 23 from 'Bossanova', 10 from 'Fiesta', 6 from 'Debora' and 3 from 'Minipaprika') were transplanted at plastic house and studied on their agronomic characters. Variation in agronomic characters was observed within the DHs of each genotype. DHs obtained from 'Derby' and 'Fiesta' exhibited wide variation in fruit yield plant^{-1} whereas averaged fruit yield plant^{-1} was highest in 'Derby' (1608 g) and less variation was observed in DHs of 'Bossanova'. Based on the agronomic characters expressed in DHs population at this environment, SP55, SP56, SP60, and SP116 from 'Special', SP8, SP10, SP14, SP16, and SP34 from 'Derby', SP115, SP119, SP142, SP143, SP196, and SP199 from 'Bossanova', SP41, SP45, and SP114 from 'Fiesta', SP21 from 'Debora' and SP91 from 'Minipaprika' identified as elite inbred lines and these DH lines could be used for commercial hybrids production in sweet pepper. Genetic relationship among the selected inbred lines using molecular markers and their response to diseases are further recommended to study.

Key words - Double haploids, Hybrids, Inbred lines, Regenerants, Yield

Introduction

Sweet pepper (*Capsicum annuum* L.) is a high value vegetable crop in Korea. It has great nutritional importance and additionally, it has offered the employment opportunity, and this crop has better export opportunity in Japanese market. Its cultivated area, productivity, and per capita consumption in Korea are increasing, however, present production of existing varieties are not enough to meet the increasing demand of domestic and international markets. Sweet pepper varieties cultivated so far in Korea have lower yielding than the imported varieties and total productivity is lower than those of the American and European countries (Shrestha and Kang, 2009). Development of higher yielding sweet pepper genotype with good quality than existing genotypes can only cope the demand of domestic and export market. Hence, it is necessary to search the rapid and efficient method and superior inbred lines for variety development on sweet pepper in Korea.

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Anther culture involves the induction of embryoid formation from immature pollen and subsequent regeneration of embryoid into plantlets. Guha and Maheswari (1964) first reported the anther culture in *Datura innoxia* for the production of haploid. Later, many studies have been carried out on anther culture in various crop species (Collins and Suderland, 1974; Hu *et al*., 1993 and Amaury-M *et al*. 1997). Variety development via double haploid system is widely used in cereal crop and in barley (*Hordeum vulgarei* L.), and 50% of cultivars in Europe are produced through double haploid system (Forster *et al*. 2007).

Wang *et al*. (1973) obtained the first in vitro haploid through anther culture. Studies have been undertaken on in vitro regeneration of chili pepper (Joshi and Kothari, 2007; Kintzios *et al*. 2001 and Prakash *et al*. 1997) and variability studies had been done on anther derived double haploids of tobacco (Deaton *et al*. 1986). Vagera (1990) observed the doubled haploid (2n) genome level as a result of chromosome double which is common in pepper cultivars. Double haploids (DHs) method using anther culture is now attracting the interest for plant breeder and geneticist. Shrestha and

Kang (2009) obtained 2.67% and 2.41% plantlets in sweet pepper cvs. Phenlene and Bossanova using anther culture, respectively and they also reported the highest percentage of chromosome doubling in 'Minipaprika'.

Anther culture technique based on DH-development is the most advanced method in pepper for new cultivar development with new fruit shape, size, color, and resistance to viruses (Caranta *et al*. 1977). DH lines obtained from heterozygous plants would shorten the time of breeding new cultivars and help to satisfy the demand of the market. Agronomic characters including plant vigor, leaf size, fruit size and yield are of special significance for breeder to choose the better parental line for heterosis breeding. Likewise, fruit selection by taste and high total soluble content is the practical method for the breeder to incorporate quality into breeding lines. Irregular fruit shape with extra large fruit is undesirable for selection whereas uniform fruit shape and size with attractive appearance are the criterion to select the variety (Greenleaf, 1986).

Larkin and Scowcroft (1981) suggested the genetic variation induced by tissue culture could be useful to plant breeding program while culture-induced variation for agronomic traits has been reported in several crop species (Larkin and Scowcroft, 1981; Orton, 1983), however, anther culture induced agronomic variation in DHs of sweet pepper genotypes has not properly studied yet. Therefore, this research was undertaken to study the agronomic variation of anther derived double haploids from sweet pepper genotypes and to identify some elite DH inbred lines for hybrid/or heterosis breeding in sweet pepper.

Materials and Methods

Source plants for haploid induction and anther culture

Flower buds of popular sweet pepper hybrids; 'Special', 'Derby', 'Bossanova', 'Fiesta', 'Debora' and 'Minipaprika' were collected from the farmer's field, Hwacheon in 2006. Unopened flower buds containing uninucleate microspores were taken for the induction of androgenesis. Flower buds containing the microspore at mid to late uninucleate stage were collected, soaked in 70% ethanol for 30 s and sterilized for 15 min with 5% sodium hypochlorite. Anthers at uninucleate stage of pollen collected from each hybrid were placed on sterilized petri-dish containing Dumas De Vaulx media, which was supplemented with 0.1 mg L^{-1} 2, 4-D, 0.1 mg L^{-1} kinetin, 3% sucrose and solidified with 0.2% phytagel. Hormone-free MS medium was composed of MS basal medium supplemented with 3% sucrose and pH 5.8. Number of anthers placed on MS basal medium from 'Special', 'Derby', 'Bossanova', 'Fiesta', 'Debora' and 'Minipaprika' were 14000, 8400, 1700, 1095, 3045, and 500, respectively. Anthers were first incubated on a medium with high concentration of growth regulators followed by the sub-cultured on medium with low amount of growth regulators. All the cultures were incubated at 35°C for one week under dark, followed by incubation at 25°C at 16 hours daylight with 20 $\mu mol.m^{-2}.s^{-1}$ of fluorescent light for 40 days.

Establishment of anther derived DHs (A-DHs) lines

Regenerated plantlets transferred to Magenta vessels containing a mixture of sterilized vermiculite, supplemented with ¼ MS inorganic salts and acclimatized under photoperiod at 25°, 20 $\mu mol.m^{-2}.s^{-1}$ of fluorescent light. Plantlets were covered with a vinyl film for the first 30 days and then transferred to 9 cm vinyl pots. Regenerated plants were transferred to pots containing Bunong horticultural nursery media (Bio-media Co. Ltd, Korea) and grown in a glasshouse with intensive care. Ploidy level was analyzed using flow cytometry (Partec GmbH Munster, Germany) in each hybrid-regenerated plantlets, and spontaneous double haploid plantlets and haploid plantlets from each hybrid were separated and grown under plastic green house. Anther derived successful double haploid populations were identified at 'Special' (32), 'Derby' (25), 'Bossanova' (23), 'Fiesta' (10), 'Debora' (6) and 'Minipaprika' (3) All DH regenerants were grown in plastic pots and fertilization, irrigation, and cultural practices were followed as the normal sweet pepper cultivation practices.

Evaluation of agronomic characters

Plant populations derived from the anther culture of each hybrid were assigned to number in SP1-SP203 (Sweet Pepper) series and all DHs of each hybrid parents were studied on their agronomic characters at Hwacheon, Kangwon-Do Province, in 2006. Plant height (cm) was measured from

the base of the plant to its top, leaf length was recorded by measuring the distance from the initiation of leaf blade along to its proximal end and leaf width was recorded by measuring the distance between two sides of a leaf blade. Fruit weight (g) was measured on digital balance, fruit length (cm) and width (cm) were measured by vernier caliper, fruit length was measured from the pedicel attachment to its apex and fruit width recorded at the basal portion of the fruits, total soluble solid (^{o}Brix) was determined by pocket refractometer pal-1 (Atago, Japan). Fruit observations were taken in randomly selected 10 fruits and fruit yield per plant was assessed by adding the weight of all consecutive fruit harvest from the plant. Data were analyzed using MS-Excel and descriptive statistic (mean, standard error, range) were used to compare the agronomic characters within the DHs.

Results

Agronomic variation in anther derived DHs

Plant and fruit characters of 32 DH populations of sweet

Table 1. Variation in agronomic characters of A-DHs of sweet pepper cv. 'Special'.

Double haploids	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Fruit weight/ fruit (g)	Fruit yield/plant (g)	Fruit length (cm)	Fruit width (cm)	TSS (^{o}Brix)	Seed/ Fruit (no.)	Fruit Shape[y]	Fruit Color[y]
SP54	53	10.3	5.9	63	1007	6.2	6.2	7.9	105	L	R
SP55	96	11.9	6.6	54	1450	7.4	5.5	8.6	87	B	R
SP56	108	12.7	7.8	16	1014	2.6	4.0	10.9	10	D	R
SP57	77	12.8	7.7	37	1083	4.4	5.1	9.6	10	D	R
SP58	46	10.9	6.2	66	1055	5.2	6.6	8.8	7	D	R
SP59	98	11.7	7.0	45	537	6.8	6.7	10.1	1	D	R
SP60	76	10.9	6.4	56	1238	8.9	4.0	9.6	62	O	R
SP62	87	10.4	7.0	65	1241	5.4	5.1	8.7	43	G	R
SP63	102	10.1	6.6	68	815	5.7	6.1	8.3	44	G	R
SP64	61	11.0	6.6	50	596	5.2	5.9	8.1	2	D	R
SP65	78	12.1	6.8	59	1130	9.1	3.7	8.3	57	O	R
SP66	82	11.6	7.2	43	905	3.8	5.9	11.7	1	D	R
SP67	98	9.0	5.4	32	1141	4.1	4.6	15.1	5	D	R
SP68	104	11.3	6.0	87	1221	6.8	6.8	9.2	50	G	R
SP69	87	11.5	6.2	28	1211	7.4	3.3	9.6	4	O	R
SP70	77	11.6	6.3	35	739	4.7	5.3	10.2	13	G	R
SP71	90	11.0	6.7	173	518	8.4	8.0	7.1	68	B	R
SP72	110	8.5	4.6	50	804	4.1	5.9	9.1	5	G	R
SP75	88	10.4	6.1	55	943	4.8	5.7	9.8	18	D	R
SP76	68	9.9	5.8	33	900	4.2	5.0	9.1	3	D	R
SP77	94	9.7	6.3	104	522	5.0	7.0	9.8	24	G	R
SP78	88	9.1	5.3	39	511	5.0	5.2	9.9	2	D	R
SP81	112	9.0	4.9	41	620	4.2	5.0	10.1	5	D	R
SP82	60	10.2	5.7	56	897	6.5	5.4	8.8	73	G	R
SP116	106	9.3	4.8	49	792	4.9	5.6	9.5	1	D	R
SP132	68	8.9	5.1	131	656	8.2	7.5	6.8	106	G	R
SP146	66	9.5	5.8	49	875	4.3	5.7	9.2	1	D	R
SP149	47	9.2	5.7	43	603	4.9	5.5	10.6	0	D	R
SP157	90	10.2	5.8	134	804	6.8	7.3	7.4	163	G	R
SP186	83	9.8	6.0	41	744	3.2	5.6	8.9	0	D	R
SP190	42	7.8	4.6	60	300	4.8	6.1	8.5	13	D	R
SP191	67	8.6	4.2	108	648	5.2	7.0	8.3	118	D	R
Mean	81.5	10.3	6.0	61.5	860.0	5.5	5.7	9.3	34.4		
SE	3.4	0.2	0.1	6.0	47.9	0.2	0.1	0.2	7.6		
Minimum	42.0	7.8	4.2	16.0	300.0	2.6	3.3	6.8	0.0		
Maximum	112.0	12.8	7.8	173.0	1450.0	9.1	8.0	15.1	163.0		

Fruit shape[y]; L = Lamuyo, B = Bell, O = Oblong, G = Glamour, Fruit color[y]; R = Red.

pepper cv. 'Special' are given in Table 1. Variation on morphological traits was observed among the double haploid populations. In plant height, average was 81.5 cm and the minimum value was in SP190 (42 cm) and maximum value was in SP81 (112 cm) followed by SP72 (110 cm), SP56 (108 cm) and SP116 (106 cm). Minimum leaf length was measured in SP190 (7.8 cm) and maximum was in SP57 (12.8 cm) followed by SP56 (12.7) with the average value was 10.3 cm. Leaf width averaged was 6.0 cm ranging from 4.2 cm (SP191) to 7.8 cm (SP56). Maximum fruit weight was obtained in SP71 (173 g) followed by SP157 (134 g) and SP132 (131 g) and minimum weight was in SP56 (16 g) with the average value of 61.5 g. Variation on fruit yield per plant was observed in the double haploid population. Average fruit yield per plant was 860 g with a minimum fruit yield per plant produced in SP190 (300 g) and maximum was in SP55 (1450 g) followed by SP62 (1241 g) and SP60 (1238 g). Fruit length was varied from 2.6 cm (SP56) to 9.1 cm (SP65) and average value was 5.5 cm whereas average fruit width was 5.7 cm with a range from 3.3 cm (SP69) to 8 cm (SP71). Average TSS (^{o}Brix) produced 9.3 with a minimum value was 6.8 (SP132) and maximum TSS obtained in SP67 (15.1) followed by SP66 (11.7), SP56 (10.9), and SP157 (10.6). Diploid line SP149 and SP186 produced seedless fruit and maximum seed number per fruit was recorded in SP157 (161) with the averaged 34.4. Out of 32 DHs, 17 lines beared dolma shaped fruits, 9 lines beared glamour shaped fruits, 3 lines beared oblong fruit shape, 2 lines beared bell shaped fruit and lamuyo shaped fruit produced in SP54. Fruit color in all the line was same as parent genotype.

Table 2. Variation in agronomic characters of A-DHs of sweet pepper cv. 'Derby'.

Double haploids	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Fruit weight/ fruit (g)	Fruit yield/ plant (g)	Fruit length (cm)	Fruit width (cm)	TSS (^{o}Brix)	Seed/ Fruit (no.)	Fruit shape^{y}	Fruit color^{y}
SP6	115	9.3	4.7	55	662	4.9	5.7	10.7	0	D	R
SP8	118	13.0	9.2	167	334	8.7	9.7	9.2	192	C	R
SP9	145	12.5	7.7	168	2351	9.0	7.3	9.4	203	B	R
SP10	134	11.5	7.7	124	2239	7.6	6.8	11.1	184	B	R
SP11	126	12.0	7.4	106	2112	7.6	6.2	9.6	221	G	R
SP12	110	13.8	9.4	121	1690	8.6	6.7	8.3	61	G	R
SP13	110	9.9	5.8	150	1950	7.7	7.3	7.5	233	G	R
SP14	152	10.9	7.4	168	4547	7.5	7.7	9.1	197	G	R
SP15	92	10.7	5.6	42	1083	4.4	4.9	8.3	53	D	R
SP16	136	13.7	8.2	130	3257	7.6	7.2	8.5	159	G	R
SP20	123	14.1	7.6	106	1795	7.0	6.7	9.3	124	G	R
SP28	136	10.7	6.5	184	1286	8.3	8.1	8.9	147	G	R
SP31	136	12.7	7.3	129	1031	8.4	6.9	9.4	191	L	R
SP32	142	12.5	7.2	175	526	7.4	7.8	9.0	236	B	R
SP33	130	13.6	7.7	171	1200	8.6	8.0	8.9	103	G	R
SP34	135	11.1	6.7	152	1819	8.8	7.4	9.1	164	G	R
SP35	100	12.0	5.7	34	34	6.0	4.5	12.7	0	G	R
SP36	138	13.2	8.2	124	1605	7.5	6.7	11.2	178	B	R
SP37	112	10.4	7.4	110	1103	6.5	6.9	8.9	60	G	R
SP38	134	12.5	6.9	170	1705	9.8	7.2	8.0	225	L	R
SP39	71	9.5	5.0	59	295	5.4	6.1	10.8	0	D	R
SP43	110	12.2	7.3	168	1514	7.9	7.9	8.5	240	B	R
SP46	100	14.4	9.7	134	1478	7.6	6.8	10.1	204	B	R
SP47	64	11.8	6.9	177	710	9.1	7.7	8.2	175	B	R
SP48	89	12.0	6.5	131	1176	6.2	7.3	8.8	126	D	R
Mean	118.3	12.0	7.1	130.2	1500.0	7.5	7.0	9.3	147.0		
SE	4.5	0.2	0.2	8.7	193.7	0.2	0.2	0.2	15.4		
Minimum	64.0	9.3	4.7	34.0	34.0	4.4	4.5	7.5	0.0		
Maximum	152.0	14.4	9.7	184.0	4547.0	9.8	9.7	12.7	240.0		

Fruit shape^{y}; D = Dolma, C = Cone, B = Bell, G = Glamour, L = Lamuyo, Fruit color^{y}; R = Red.

DHs obtained from anther culture and their morphological variations are given in Table 2. Average plant height was 118.3 cm with ranged from 64 cm (SP47) to 152 cm (SP114). Leaf length varied from 9.3 cm (SP6) to 14.4 cm (SP46) with averaged 12 cm. Minimum leaf width recorded in SP6 (4.7 cm) and maximum measured in SP46 (9.7 cm) with the mean leaf width of 7.1. Similarly, average of fruit weight per fruit and fruit yield per plant were 130.2 g and 1500 g, respectively. The highest fruit weight per fruit and yield per plant produced in SP28 (184 g) and SP14 (4547 g), respectively. Average fruit length was 7.5 cm ranging from 4.4 cm (SP15) to 9.8 cm (SP38). The highest fruit width was produced in SP8 (9.7 cm) and the lowest in SP35 (4.5 cm) and average fruit width was 7 cm. The highest TSS (^{o}Brix) was measured in SP35 (12.7) followed by SP36 (11.2) and SP10 (11.1). SP6, SP35 and SP39 were produced seedless fruit and maximum seed produced in SP43 with the average of 147. Out of 25, 12 lines were identified as glamour shaped fruits, 7 lines were bell shaped, 4 lines were dolma shaped and 2 lines identified as lamuyo shaped fruit and no variation was observed in fruit color.

Agronomic characters of 23 A-DHs populations derived from cv. 'Bossanova' and their variation are illustrated in Table 3. Plant height averaged 68.3 ranging from 29 cm (SP83) to 127 cm (SP109). Average leaf length and leaf width were 8.4 cm and 5.1 cm, respectively. The highest fruit weight per fruit was measured in SP142 (119 g) and the lowest in SP161 (23 g) with averaged 59.5 g. Fruit yield per plant was varied from 314 g (SP156) to 1179 g (SP196) with the average 673.2. Fruit length was ranged from 3.1 cm (SP115) to 7.8 cm (SP101) and average value was 5.1 whereas maximum fruit width was recorded in SP142 (7.4 cm) and

Table 3. Variation in agronomic characters of A-DHs of sweet pepper cv. 'Bossanova'.

Double haploids	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Fruit weight/ fruit (g)	Fruit yield/ plant (g)	Fruit length (cm)	Fruit width (cm)	TSS (^{o}Brix)	Seed/ Fruit (no.)	Fruit shape[y]	Fruit color[y]
SP83	29	8.4	5.3	79	316	7.1	6.1	9.0	67	D	Y
SP84	65	8.9	6.2	38	803	3.3	5.6	8.2	13	D	Y
SP85	44	11.0	6.9	31	756	4.1	4.7	9.1	1	D	Y
SP101	38	7.6	5.1	63	876	7.8	5.5	7.7	58	G	Y
SP104	74	8.1	5.5	56	730	6.9	5.4	9.1	0	D	R
SP109	127	9.2	5.4	69	695	4.1	6.5	7.8	42	D	R
SP115	112	9.1	5.6	28	533	3.1	4.6	9.4	2	D	R
SP119	65	7.4	4.8	82	990	6.0	6.2	10.2	74	D	Y
SP120	78	9.1	5.4	45	857	4.2	5.5	8.9	0	D	Y
SP134	92	10.0	7.2	72	720	4.5	6.1	7.8	16	D	Y
SP137	58	8.2	5.0	35	461	3.7	5.3	9.5	4	D	Y
SP142	42	7.1	5.0	119	596	6.3	7.4	6.8	162	D	Y
SP143	64	7.6	4.8	72	576	7.1	5.7	8.2	70	D	Y
SP145	60	9.9	5.5	86	687	5.9	6.4	8.7	86	D	Y
SP156	73	7.9	4.4	39	314	4.6	4.7	7.8	13	C	Y
SP161	72	6.4	3.9	23	578	3.1	4.3	8.6	3	D	Y
SP164	57	8.7	4.3	77	848	5.8	6.0	8.7	64	D	Y
SP192	85	7.3	4.1	27	760	3.4	4.7	9.1	0	D	Y
SP193	50	7.4	3.8	58	407	4.9	6.0	9.5	0	D	Y
SP196	94	8.8	5.8	79	1179	5.3	6.7	9.6	1	D	Y
SP197	56	8.4	5.4	70	488	4.8	6.1	7.7	101	D	Y
SP198	83	9.1	4.7	69	689	6.2	5.8	8.0	34	G	Y
SP199	55	7.5	5.2	52	626	5.1	5.7	10.2	25	G	Y
Mean	68.3	8.4	5.1	59.5	673.2	5.1	5.7	8.3	36.3		
SE	4.7	0.2	0.1	4.9	43.8	0.2	0.1	0.4	8.8		
Minimum	29.0	6.4	3.8	23.0	314.0	3.1	4.3	7.7	0.0		
Maximum	127.0	11.0	7.2	119.0	1179.0	7.8	7.4	10.2	162.0		

Fruit shape[y]; D = Dolma, G = Glamour, C = Cone Fruit color[y]; Y = Yellow, R = Red.

minimum in SP161 (4.3 cm) with averaged of 5.7. Soluble solid produced the highest in 10.2 (SP119) followed by SP199 (10.2) and the lowest in SP101 (7.7) and SP197 (7.7). SP104, SP120, SP192 and SP193 produced the seedless fruit whereas maximum seed was produced in SP142 (162). SP101, SP198 and SP199, and SP156 were observed glamour and cone shaped fruit, respectively and rests of the tested lines were found dolma shaped fruit. SP104, SP109, and SP115 were red colored fruit and remaining 20 DHs were yellow colored fruit.

A-DHs from cv. 'Fiesta' and agronomic characters are presented in Table 4. Average plant height was 102.8 cm, with minimum in SP95 (70 cm) and maximum in SP50 (130 cm) followed by SP26 (128 cm). Average leaf length and leaf width were 12.7 cm and 6.6 cm, respectively. The highest leaf length and width were produced in SP45 (18.1 cm) and SP45 (9.9 cm), respectively. Fruit weight per plant averaged 88.5 g, with minimum weight produced in SP114 (29 g) and maximum weight in SP51 (148 g) followed by SP27 (144 g). Fruit yield per plant averaged 791.1 g, where the highest yield per plant was measured in SP45 (2482 g) and the lowest in SP41 (116 g). Fruit length was varied from 8.9 cm (SP45) to 3.0 cm (SP114) with averaged 5.9 and fruit width was averaged 6.2 where minimum and maximum width were produced in SP41 (4.6 cm) and SP51(7.5 cm), respectively. SP41 gave maximum (11) soluble solid (^{o}Brix) followed by SP114 (10.4) and SP26 (10.1) where minimum was in SP27 (7.1). SP50 gave seedless fruit and maximum seed produced in SP51 (215). Shape of

Table 4. Variation in agronomic characters of A-DHs of sweet pepper cv. 'Fiesta'.

Double haploids	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Fruit weight/ fruit (g)	Fruit yield/ plant (g)	Fruit length (cm)	Fruit width (cm)	TSS (^{o}Brix)	Seed/ Fruit (no.)	Fruit Shape[y]	Fruit color[y]
SP26	128	12.9	5.7	42.6	511	5.2	5.1	10.1	6	R	Y
SP27	126	15.3	7.2	144	1007	8.4	7.3	7.1	117	L	Y
SP41	82	12.3	5.6	39	116	4.7	4.6	11.0	5	D	Y
SP42	105	12.8	5.6	66.2	662	4.3	5.8	8.0	3	R	Y
SP45	106	18.1	9.9	124	2482	8.9	6.7	8.2	116	L	R
SP50	130	13.6	7.3	107	215	7.3	6.5	8.0	0	G	Y
SP51	105	14.3	8.3	148	1185	7.4	7.5	8.9	215	D	Y
SP95	70	9.9	5.5	89	625	5.7	6.7	7.5	51	D	Y
SP114	88	9.4	5.3	29	817	3.0	4.8	10.4	0.1	D	Y
SP203	88	9.3	5.6	97	291	5.0	7.4	8.8	2	D	Y
Mean	102.8	12.7	6.6	88.5	791.1	5.9	6.2	8.0	51.5		
SE	6.5	0.8	0.4	13.7	216.5	0.6	0.3	0.9	23.4		
Minimum	70.0	9.3	5.3	29.0	116.0	3.0	4.6	7.1	0.0		
Maximum	130.0	18.1	9.9	148.0	2482.0	8.9	7.5	11.0	215.0		

Fruit shape[y]; R = Round, L = Lamuyo, D = Dolma, Fruit color[y]; Y = Yellow, R = Red.

Table 5. Variation in agronomic characters of A-DHs of sweet pepper cv. 'Debora'.

Double haploids	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Fruit weight/ fruit (g)	Fruit yield/ plant (g)	Fruit length (cm)	Fruit width (cm)	TSS (^{o}Brix)	Seed/ Fruit (no.)	Fruit shape[y]	Fruit color[y]
SP21	132	12	7	143	2137	9.1	6.8	8.9	127	B	R
SP22	120	13.1	6.6	175	1053	9.0	8.0	8.0	166	B	R
SP23	99	13.4	7.7	112	559	5.1	7.0	8.0	122	D	R
SP24	110	9.9	5.5	152	1524	8.1	7.9	8.4	180	L	Y
SP25	117	11.8	6.8	195	2145	8.9	8.1	7.9	222	D	R
SP30	136	12.8	8.1	149	2235	8.1	7.1	9.8	177	B	R
Mean	119.0	12.2	7.0	154.3	1608.8	8.1	7.5	7.2	165.7		
SE	5.6	0.5	0.4	11.6	281.5	0.6	0.2	1.5	15.2		
Minimum	99.0	9.9	5.5	112.0	559.0	5.1	6.8	7.9	122.0		
Maximum	136.0	13.4	8.1	195.0	2235.0	9.1	8.1	9.8	222.0		

Fruit shape[y]; B = Bell, D = Dolma, L = Lamuyo, Fruit color[y]; R = Red, Y = Yellow.

the fruits noted in all the DHs were round, lamuyo, dolma and glamour and fruits of SP45 red colored and rests of the lines were noted yellow colored fruit.

Six A-DHs from cv 'Debora' and their agronomic variation were analyzed and given in Table 5. Average plant height was 119 cm, ranging from 99 cm (SP23) to 136 cm (SP30). Leaf length was varied from 9.9 cm (SP24) to 13.4 cm (SP23) with averaged 12.2 cm whereas lead width was varied from 5.5 cm (SP24) to 8.1 cm (SP30) with an averaged 7.0 cm. SP25 yielded maximum (192 g) fruit weight per plant followed by SP22 (175 g) and minimum produced in SP23 (112 g) and averaged value was 154.3 g. Average fruit yield per plant was 1608.8 g and the highest yield produced in SP30 (2235 g) followed by SP25 (2145 g) and SP21 (2137 g), and the lowest in SP23 (559 g). Fruit length was varied from 5.1 (SP23) to 9.1 (SP21) with averaged 8.1 cm whereas fruit width averaged 7.5 cm ranging from 6.8 cm (SP21) to 8.1 cm (SP25). Total soluble solid was varied among the DH lines and ranged from 7.9 to 9.8, the highest TSS was recorded in SP30 (9.8) and the lowest in SP25 (7.9) with averaged of 7.2. Seeds number produced the highest in SP25 (222) and the lowest in SP23 (122) where average value was 165.7. Bell, dolma and lamuyo shaped fruits were identified on six DH populations of 'Fiesta' genotype and except SP24 (yellow); all lines were red colored fruits.

Agronomic variation of A-DH lines derived from cv. 'Minipaprika' is mentioned in Table 6. Average of plant height was 78 cm, and highest leaf length and width (6.5 cm) were measured in SP91. Likewise, the highest fruit weight (18 g) per fruit and fruit yield (663 g) per plant were also recorded in SP91 and no variation was existed in fruit length, fruit width and total soluble content, and higher (>10.0) TSS content was noted in all the DH lines. SP91 and SP155 were observed glamour shaped fruit, SP133 was cone shaped, fruit color of SP91 was orange, and other two lines were red colored fruit.

Agronomic variation of A-DHs within the genotypes

Average performance of agronomic traits in all the DH regenerants of sweet pepper genotypes are explained in

Table 6. Variation in plant and fruit characters of A-DHs of sweet pepper cv. 'Minipaprika'.

Double haploids	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Fruit weight/ fruit (g)	Fruit yield/ plant (g)	Fruit length (cm)	Fruit width (cm)	TSS (°Brix)	Seed/ Fruit (no.)	Fruit Shape[y]	Fruit color[y]
SP91	80	11.5	6.5	18	663	4.4	3.4	10.2	10	G	O
SP133	88	7.6	4.2	11	624	4.5	2.6	10.0	6	C	R
SP155	66	5.3	2.9	12	411	4.0	3.2	10.0	117	G	R
Mean	78.0	8.1	4.5	13.7	566.0	4.3	3.1	10.1	44.3		
SE	6.4	1.8	1.0	2.1	78.3	0.1	0.2	0.1	6.3		

Fruit shape[y]; C = Cone, G = Glamour, Fruit color[y]; O = orange, R = Red.

Table 7. Mean (Mean ± SE) of agronomic characters of A-DHs of sweet pepper genotypes.

Characters	Genotypes					
	'Special'	'Derby'	'Bossanova'	'Fiesta'	'Debora'	'Minipaprika'
Plant height (cm)	81.5 ± 3.4[y]	118.3 ± 4.5	68.3 ± 4.7	102.8 ± 6.5	119.0 ± 5.6	78 ± 6.4
Leaf length (cm)	10.3 ± 0.2	12.0 ± 0.2	8.4 ± 0.2	12.7 ± 0.8	12.2 ± 0.5	8.1 ± 1.8
Leaf width (cm)	6.0 ± 0.1	7.1 ± 0.2	5.1 ± 0.1	6.6 ± 0.4	7.0 ± 0.4	4.5 ± 1.0
Fruit weight/fruit (g)	61.5 ± 6.0	130.2 ± 8.7	59.5 ± 4.9	88.5 ± 13.7	154.3 ± 11.6	13.7 ± 2.1
Fruit yield/plant (g)	860 ± 47.9	1500.0 ± 193.7	673.2 ± 43.8	791.1 ± 216.5	1608.8 ± 281.5	566 ± 78.3
Fruit length (cm)	5.5 ± 0.2	7.5 ± 0.2	5.1 ± 0.2	5.9 ± 0.6	8.1 ± 0.6	4.3 ± 0.1
Fruit width (cm)	5.7 ± 0.1	7.0 ± 0.2	5.7 ± 0.1	6.2 ± 0.3	7.5 ± 0.2	3.1 ± 0.2
TSS (°Brix)	9.3 ± 0.2	9.3 ± 0.2	8.3 ± 0.4	8.0 ± 0.9	8.5 ± 1.5	10.1 ± 0.1
Seed/fruit (no.)	34.4 ± 7.6	147 ± 15.4	36.3 ± 8.8	51.5 ± 23.4	165.7 ± 15.2	44.3 ± 6.3

[y]mean ± SE.

Table 8. Variability range of agronomic characters of A-DHs of sweet pepper genotypes.

Characters	Genotypes					
	'Special'	'Derby'	'Bossanova'	'Fiesta'	'Debora'	'Minipaprika'
Plant height (cm)	42-112	64-152	29-127	70-130	99-136	66-88
Leaf length (cm)	7.8-12.8	9.3-14.4	6.4-11.0	9.3-18.1	9.9-13.4	5.3-11.5
Leaf width (cm)	4.2-7.8	4.7-9.7	3.8-7.2	5.3-9.9	5.5-8.1	2.9-6.5
Fruit weight/fruit (g)	16-173	34-184	23-119	29-148	112-195	11-18
Fruit yield/plant (g)	300-1450	34-4547	314-1179	116-2482	559-2237	411-663
Fruit length (cm)	2.6-9.1	4.4-9.8	3.1-7.8	3.0-8.9	5.1-9.1	4.0-4.4
Fruit width (cm)	3.3-8.0	4.5-9.7	4.3-7.4	4.6-7.5	6.8-8.1	2.6-3.4
TSS (^{o}Brix)	6.8-15.1	7.5-12.7	7.7-10.2	7.1-11.0	7.9-9.8	10.0-10.2
Seed/fruit(no.)	0.0-163.0	0.0-240	0.0-162.0	0.0-215	122-222	6-117

Table 7. The highest plant height was obtained in the DH of 'Debora' (119 cm) followed by the DH lines of 'Derby' (118.3 cm) and the lowest in Bossanova (68.3 cm). Leaf length averaged was highest in 'Fiesta' (12.7 cm), followed by 'Debora' (12.2 cm) and 'Derby' (12 cm) and average leaf width was highest in the regenerants of 'Derby' (7.1 cm) followed by 'Debora' (7 cm). DHs of 'Debora' yielded the highest (154.3 g) fruit weight per fruit followed by 'Derby' (130.2 g) whereas the least fruit weight was accounted in 'Minipaprika' (13.7 g). Average fruit yield per plant was obtained highest in DH lines of 'Debora' (1608 g) and 'Derby' (1500 g). Similarly, average fruit length and fruit width were measured highest (8.1 and 7.5 cm) in DH of 'Debora'. Total soluble (^{o}Brix) content was highest in DH of 'Minipaprika' (10.1). Average seed number was also highest in DH of 'Debora' (165.7) and 'Derby' (147) than other genotypes.

Variability ranges of all agronomic traits in all DH regenerants of each genotype are mentioned in Table 8. Wide variation in plant height was recorded in DH of 'Bossanova' (29 ~ 127 cm) and small variation was in 'Minipaprika' (66 ~ 88 cm). Variation in leaf length in DH population of 'Fiesta' was highest (9.3 ~ 18.1 cm) whereas high leaf width variation was in DHs of 'Derby' (4.7 ~ 9.7 cm). Wide variation in fruit weight (34 ~ 184 g) per fruit and fruit yield (34 ~ 4513 g) per plant were existed in the diploid population of 'Derby' followed by 'Fiesta'. Fruit length range was highest in 'Special' (2.6 ~ 9.1 cm) whilst the highest fruit width range was in 'Derby' (4.5 ~ 9.7 cm). Total soluble content was varied among the DH of each genotype and this range was highest in 'Special' (6.8-15.1) and the lowest in 'Minipaprika' (10-10.2). Likewise, seed was obtained highest in 'Derby' (240) followed by 'Fiesta' (215).

Discussion

This study examined the variability in agronomic characters within the double haploid regenerants of each sweet pepper genotype and compared the variability of traits among the genotypes. A-DHs within each sweet pepper genotype exhibited great variation in plant height, leaf size, fruit yield, TSS, fruit shape, and fruit color in the DH of 'Bossanova', 'Fiesta', 'Debora' and 'Minipaprika', Valadez-Bustos *et al*. (2009) has reported higher width and weight of fruit in the regenerated plants of Serrano type, and they observed the heavier fruit with larger diameters in the micropropagated plants of Serrano type, and found the significant yield difference per plant in the regenerated plants of Jalapeno and Serrano types. In this study, differences in fruit yield of each DH regenerants of genotype are observed and this ample agronomic variability observed in DH populations might be useful to breeder for selecting the better-inbred lines for hybrid breeding. In this work, differences in agronomic performance among the regenerated plant were observed but the agronomic variation depended on the DH lines of genotype tested. Yield variation was high at DH inbred of 'Derby' and 'Fiesta' than other genotypes and that might be due to genetic make of the genotype. Brown and Wernsman (1982) reported that genetic differences between double haploid lines and their source cultivar are largely due to their nuclear origin. They also

reported that genetic variation among the androgenetic doubled haploid lines in tobacco caused by mutations in nuclear genes during anther culture. Deaton *et al*. (1986) mentioned that anther derived lines exhibited greater variation than the sexual materials. It can be concluded that variation occurred in DH lines of each sweet pepper genotype might be due to the heterozygosity in the original varietal material. Moreover, differences within the DH lines derived from the same parents might be due to the result of naturally produced variation and mutations during anther culture. Variability of anther-derived doubled haploid lines has been attributed to residual heterozygosity in a source plant for anther culture (Deaton *et al*. 1982) and mutations during anther culture (Oinuma and Yoshida, 1974). Burk and Matzinger (1976) observed much variability in the spontaneous DH lines than colchicines treated diploid plants. Deaton *et al* (1982) reported minimal alterations in doubled-haploid lines from inbred cultivars in tobacco. Kumashiro and Oinuma (1986) had found that spontaneous-DHs exhibited great quantitative variability within the family in tobacco as colchicines-DH.

Few molecular studies have dealt the variability among the A-DH lines in sweet pepper. Amzad et al (2003) had studied the genetic and morphological variation in R_0 and R_1 chili pepper plants regenerated from tissue culture. Gyulai *et al*. (2000) reported that high level of genetic diversity among DH-R_2 plants using PCR analysis in sweet pepper. From this discussion and overall, it can be said that chromosomal alterations, cytoplasmic effects, residual heterozygosity in the parents genotype, mutation during anther culture, development of altered genomes in the microspores population, and somaclonal variation are attributed to create the variation in A-DH lines of sweet pepper genotypes.

Conclusion

In this research, agronomic characters of anther derived DH lines from different sweet pepper genotypes were studied and variation in the agronomic characters was observed in all the A-DH lines of each genotype. Variability of characters in DHs population within the same parent due to their naturally produced variation and magnitude of variation differed in DHs of genotype being tested. Overall, residual heterozygosity was existed in the parental source of anther culture; mutations during the anther culture and some cytoplasmic effect in the microspore culture are the fundamental reasons causing the variation among the DH lines. Fruit weight and fruit yield variation were highest in DHs of 'Derby' and breeder could select the outstanding DH lines among the population for further variety improvement. Variation of fruit yield per plant, pericarp thickness, total soluble solid, fruit shape and size are of special significance for breeder to select the inbred lines. Big size fruits are undesirable since it is associated with lower productivity and irregular fruit shape and poor quality. Based on agronomic characters expressed in DH lines, SP55, SP56, SP60, and SP116 from 'Special', SP8, SP10, SP14, SP16, and SP34 from 'Derby', SP115, SP119, SP142, SP143, SP196, and SP199 from 'Bossanova', SP41, SP45, and SP114 from 'Fiesta', SP21 from 'Debora' and SP91 from 'Minipaprika' are identified as elite inbred lines and these DH lines could be used for commercial hybrids production in sweet pepper. Disease responses of selected inbred lines are missed in this study and also genetic distance estimation among selected inbred lines using molecular markers are further recommended to study.

Acknowledgement

This research was performed in the support of Korean Research Foundation Grant under Korean Government (MOEHRD, Basic Research Promotion Fund, KRF-2006-331-F00019).

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(Received 25 May 2010 ; Accepted 22 November 2010)