

Gametoclonal and Somaclonal Variation among Head Cabbage Androgenic Lines of R_1 and R_2 Generations Obtained from Jaguar F_1 Hybrid

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Abstract

Head cabbage androgenic genotypes of R_1 and R_2 generations derived from Jaguar F_1 donor hybrid were evaluated according to their agroeconomical characters, ability for generative propagation, self-incompatibility, intraline uniformity and stability of morphological characters. Most of R_1 and R_2 genotypes had desired morphological characters. However, in R_1 generation 21 lines showed lack of internal uniformity probably due to the somaclonal variation. Genotypes of R_1 and R_2 generation from different embryos were more diversified than genotypes derived from the same embryo according to self-incompatibility, ability for seed setting, vegetative period, head shape, internal sump length and mass of head. Rigid selection of androgenic material at R_0 , R_1 and R_2 pedigree according to absence of somaclonal variation, agroeconomical traits, self-compatibility and seed setting ability seemed to be crucial for the deriving stable and suitable for the breeding genotypes of head cabbage.

Keywords: Head cabbage, Androgenic lines, Morphological characters, Internal uniformity, R_1 and R_2 generation

1. Introduction

Obtaining of androgenic plants from Brassica crops as cabbage, broccoli, cauliflower and Brussels sprout by the use of anther culture technique is widely used as a source of desired genetic diversity (Chiang *et al.* 1985, Ockendon 1986, 1988, Dore & Boulidard 1988, Chauvin *et al.* 1993, Górecka *et al.* 1997, Farnham 1998, Kamiński *et al.* 1999, Wang *et al.* 1999, Kamiński *et al.* 2004, 2005). Androgenic plants with their genetic variation from gametic cells of donor cultivars represent a gametic array each having a different contribution from the parents (Morisson & Evans, 1988). However, anther and microspore cultures are rarely used in cabbage breeding programs probably due to the difficulty in obtaining of androgenic lines with good quality, uniformity and stability in consecutive generations. There are also very few reports with description of practical use of androgenic cabbage genotypes for the creation of commercial cultivars and F_1 hybrids and it practically impossible to determine which cabbage cultivars are based on androgenic parents. Low quality of R_0 androgenic plants and their poor agronomic performance might be caused by the lack of natural selection in the first stages of haploid development as well as by the gametoclonal and somaclonal variation generated itself in plant cell culture (Larkin & Scowcroft 1981). Somaclonal variation that took place during recovery process from *in vitro* culture, leads to the creation of additional genetic variability with crop improvement potential (Brown & Thorpe, 1995, Evans 1989), but could have a negative effect on the agronomic performance of DH plants, their genetic stability and differentiated ability to generative propagation. This can be the reason why doubled haploid (DH) lines often appear to be inferior in comparison to conventionally obtained inbred lines. Therefore to avoid the undesired source of diversity androgenic material should be multiplied vegetatively or generatively from every single plant (Niemirówicz-Szczyt 1997). Diversified level of intraline uniformity among androgenic Brussels sprout lines of R_2 generation was described by Kamiński (2008), however, the quality and internal uniformity were relatively better than in R_0 and R_1 androgenic populations from Philemon F_1 hybrid (Kamiński 2004, 2005). Head cabbage is characterized by biennial habit with vernalization period from 8 to 12 weeks, strong depression of inbred lines and usually exhibits diversified and/or high level of self-incompatibility (Thompson 1957, Ockendon 1973, Wallace 1979, Hoser-Krauze 1993, Kamiński 2000, 2001). Cabbage F_1 hybrids are heterozygous according to self-incompatibility and possessed two different s-alleles with four possibly types of domination in pollen and pistil (Haruta 1962, Takayama & Isogai 2003, Stephenson *et al.* 1999, Schopfer *et al.*

1999, Hatakeyama *et al* 2001). Obtaining seeds of cabbage androgenic R₁ populations is one of the most difficult step, as DH genotypes usually give very low seed yields in comparison with the lines derived from classical selection (Chauvin *et al.* 1993, Farnham 1998, Kamiński *et al* 2004, 2005.).

The aim of this study was to evaluate the influence of gametoclonal and somaclonal variation on agromorphological traits, intraline uniformity, ability for generative propagation and level of self incompatibility of head cabbage androgenic R₁ and R₂ genotypes obtained from highly embryogenic 'Jaguar F₁' hybrid both in vegetative and generative stages of development.

2. Material and methods

Androgenic head cabbage plants were obtained from Jaguar F₁ hybrid by the use of anther culture at the Research Institute of Vegetable Crops, Skierniewice, Poland in 2004. Regenerated 103 DH plants of R₀ generation derived from 11 androgenic embryos (Table 1) identified as diploids by the use of chromosome count and flow-cytometry analysis were planted into 3 l. plastic pods and cultivated in the greenhouse. Fertilization, watering and plant protection against pests and diseases were provided according to current recommendations for that species. R₀ genotypes in the stage of 12 true leaves were vernalized from November 2004 until the end of March 2005 in temperature 4-7°C. Seed stalks were isolated with the covers made from paper and plastic to avoid undesired cross pollination by insects. R₀ plants were individually self pollinated by hand in open flower and bud stage at the 20 green buds/flowers to obtain seeds of R₁ generation. Matured silicas were harvested 90 days after pollination, dried, counted and seeds were extracted separately for each of plant. Seeds of 39 R₁ genotypes developed from 8 embryos were selected for the evaluation at the field of Research Institute of Vegetable Crops in 2006.

After evaluation at the field, single plants of R₁ generation with desired agroeconomical characters from internally uniform genotypes were assigned for the generative propagation. For each of selected R₁ genotype ten cuttings were harvested, transplanted, rooted and vernalized. In 2007 twelve R₁ populations obtained from seven androgenic embryos, developed seed stacks and were self pollinated in the greenhouse at open flower and green bud stage. Eight R₁ populations (2.1/31, 2.2/4, 2.2/27, 2.3/14, 2.3/8, 4.2/14, 6.2/7, 6.5/7) were also propagated in 9 m² field cages by the use of bees (*Osmia rufa*) (Table 1). In each cage five vernalized plants of the single androgenic line were planted. Seeds of consecutive R₂ generation were harvested, dried and extracted separately for each of genotype. In 2008 eighteen androgenic genotypes of R₂ generation (twelve obtained in the greenhouse, six from field cages) were evaluated at the field at Research Institute of Vegetable Crops, Skierniewice according to agroeconomical characters. Plants of R₁ (2006) and R₂ (2008) generations developed from seeds in the greenhouse in mid-April. One-month-old seedlings were planted in the field (spacing 50 x 60 cm) in a completely randomized block design with three replications. Each plot consisted of ten plants in one row. The soil type was a pseudopodsolic over loamy sand (1,5 % organic matter, pH 6.5). Fertilization, pest and disease control followed the current recommendations. Plants were harvested gradually from the beginning of August to the end of September when heads reached maturity. Mass, length and width of head and the internal stump length were measured and the head shape (length / width) and internal stump (internal stump length / head height) coefficient were calculated. As a control Jaguar F₁ hybrid was used. Results were subjected to an analysis of variance (ANOVA). The significance of differences among means was evaluated by Newman-Keul's test at $\alpha = 0,05$. Other morphological characteristics of androgenic population such as: intraline uniformity and length of vegetation period from planting to harvest, were classified separately for each plot.

3. Results and discussion

3.1

Seed productivity of R₀/R₁ head cabbage androgenic population obtained from Jaguar F₁ hybrid was diversified and ranged from 0.1 seeds/silica for line 2.3/14 to 6.15 seeds/silica for 4.2/15 line after pollination at the bud stage (Table 2). The average seed setting at bud pollination for all genotypes (2.42 seed/silica) was five-fold higher than for open flower pollination (0.53 seed/silica). The average seed set of six genotypes from 4,2 embryo was higher both in bud (4.0 seed/silica) and in open flower stage (1.09 seed/silica), than from other androgenic embryos. Eight genotypes (2.2/4, 2.2/7, 2.2/27, 2.3/8, 4.2/4, 4.5/25, 6.5/1, 6.5/2) did not set seeds after open flower pollination according to the high level of self incompatibility. For all 10 androgenic lines resulted from 6,5 embryo, bud pollination was 10 fold more effective (2.59 seed/silica) than after pollination at opened flower stage (0.22 seed/silica). The ability of seed propagation of androgenic R₁ genotypes from the same embryo was also highly diversified. The highest differences in seed set effectiveness for androgenic genotypes obtained from 4,2 embryo ranged from 0.00 seeds/silica for line 4.2/4 to 3.00 seed/silica for line 4.2/14 when pollinated at open flower stage. The highest differences in seed setting after bud pollination was observed for 2,1 embryo between

plant 2.1/12 (0.39 seed/silica) and 2.1/33 (6.00 seed/silica). Androgenic R₁ generation, was also characterized by differentiated ability of seed setting in 2007 (Table 3). In the greenhouse, seed setting of R₁/R₂ pedigree ranged from 0.05 seed/silica (6.5/7 line) to 1.25 seed/silica (2.2/7 line) for open pollination and from 0.27 seed/silica (2.2/15 line) to 3.14 seed/silica (2.1/31 line) for bud pollination. Two androgenic lines (2.1/31, 2.2/27) propagated at the field, that did not set seeds, were characterized by the high level of self-incompatibility. Two lines (6.2/7, 4.2/14) that set the highest mass of seeds (4.2 g, 2.6 g/plant) were self compatible. Four genotypes (2.2/4, 2.3/14, 2.3/8, 6.5/7) set the average seed yield from 0.1 to 0.5 g/plant and were partially self-compatible.

In both years of field experiment (2006, 2008), androgenic genotypes of R₁ generation and 16 androgenic R₂ lines had lower head mass than cv. 'Jaguar F₁' probably according to inbreeding depression (Table 4, 5). Only in 2008 two lines of R₂ populations: 2.2/4 (3.48 kg) and 2.1/31 (3.58 kg) had similar yield to 'Jaguar F₁' (3.22 kg). Androgenic R₁ lines were not significantly diversified as to the mass of head, with the exception of 4.5/25 line (2.47 kg) that yielded better than 4.2/17 (1.47 kg). More significant differences according to head mass between androgenic genotypes of head cabbage were observed in R₂ generation as to inbreeding depression probably. Three androgenic R₂ lines (2.2/4, 6.2/7, 4.2/14), propagated by sib-pollination in the field cages, yielded better than those propagated by self pollination in the greenhouse. Two other lines 6.5/7 and 2.3/8 had higher mass of head when self pollinated at the greenhouse and for 2.3/14 line the mass of head were similar for both methods of propagation (Table 5).

The head shape coefficient of androgenic R₁ lines ranged from flattened 0.81 (line 6.5/19) to elongated 1.2 (line 4.2/4) (Table 3). R₁ genotypes from the same embryo had similar head shape and did not differed significantly from each other according to that trait. Genotypes from 6.5 and 5.2 embryos had more flattened shape of head (0.81-1.0) than genotypes derived from 4.2 and 4.5 embryos (1.02-1.2), while genotypes from 2.2, 13.1 and 2.1 embryo were rounded (0.97-1.11) similarly to Jaguar F₁ donor cultivar. In consecutive R₂ generation, head cabbage lines were more uniform according to that trait as most genotypes were rounded and were similar to 'Jaguar F₁' or slightly elongated. Only 4.2/14 and 4.2/14i R₂ genotypes were significantly more elongated (1.29, 1.33) than all other populations. (Table 5). The way of propagation of R₂ androgenic lines had no influence on the head shape.

Internal stump length coefficient of R₁ genotypes ranged from 0.37 (6.5/2 line) to 0.58 (6.5/19 line). Two androgenic R₁ lines (6.5/19, 2.1/16) had significantly longer internal stump (0.58, 0.56 respectively) than their donor cultivar (0.44), while internal stump coefficient of other genotypes were at the similar level. Two lines (6.5/7, 6.2/7) of R₂ generation had significantly longer internal stump (0.56, 0.54) than Jaguar F₁ (0.45).

21 lines of androgenic R₁ generation were not uniform according to several other morphological characters such as waxiness, color and blistering of leaves, earliness and shape of head. Twelve from 39 R₁ genotypes were characterized by the lack of intraline uniformity according to more than one trait, nine lines were not uniform according to one of investigated characters and only nineteen R₁ lines evaluated at the field were internally uniform (Table 4). In contrary to R₁ generation, androgenic R₂ lines were generally characterized by internal uniformity with the exception of 6.2/7 genotype (Table 4).

In both years of field experiments (2006, 2008) androgenic pedigree were more diversified than Jaguar F₁ hybrid according to vegetation period. Generally, head cabbage genotypes obtained from the same embryo were characterized by similar vegetation period, while differences between lines from different embryos were observed both in R₁ and R₂ populations. Five R₁ lines 2.2/4, 2.2/7, 2.2/14, 2.2/19 and 2.3/14 was the earliest from all genotypes and harvested 70 days after planting (Table 3). Fourteen R₁ lines (2.1/14, 2.1/16, 2.1/31, 2.1/33, 2.2/15, 2.2/16, 2.2/27, 2.3/5, 2.3/6, 2.3/7, 2.3/8, 2.2.3/10, 3/12, 2.3/17) were also earlier than 'Jaguar F₁' with vegetation period from 75 to 80 days while other R₁ genotypes yielded after 90-95 days when planted at the field. Vegetation of R₂ androgenic head cabbage lines in 2008 was more extended and lasted from 80 to 110 days from planting to harvest (Table 5). Eleven genotypes (2.1/31, 2.2/4, 2.2/4i, 2.2/7, 2.2/15, 2.2/27, 2.3/8, 2.3/8i, 2.3/14, 2.3/14i, 2.3/17) had shorter than 'Jaguar F₁' vegetation period (80 days), three lines (4.2/14, 4.2/14i, 6.5/7), were similar to donor cultivar (95-100 days) and four lines (6.2/7, 6.2/7i, 6.5/7, 13.1/1) had the longest vegetation from all accessions (110 days) (Table 5).

3.2

Smaller head mass of androgenic R₁ and R₂ lines in comparison to Jaguar F₁ donor cultivar, confirmed the presence of strong inbreeding depression, characteristic for androgenic lines of cabbage obtained from 'Kamienna Głowa' (Kamiński 2000) and for traditionally derived inbred genotypes. Relatively lower ability for seed propagation both for bud and open-flower pollination among androgenic genotypes obtained from Jaguar F₁ hybrid in comparison to traditionally derived inbred lines described for cabbage plants by Dickson and Wallace

(1986), can be explained not only by the occurrence of inbreeding depression typical for DH lines but also by the somaclonal variation. Difficulties with generative propagation of androgenic Brussels sprout genotypes described by Kamiński *et al.* (2005) may decrease the effectiveness of anther culture as a source of new genetic diversity. Low number of R_1 seeds obtained from doubled haploid plants showed that somaclonal variation in R_0 population critically affected the ability for generative propagation of androgenic head cabbage plants from Jaguar F_1 hybrid.

Lack of intraline uniformity among most of androgenic R_1 lines was probably caused by somaclonal variation that also affect negatively the quality of DH lines (Brown & Thorpe 1995, Larkin & Scowcroft 1981, Niemirowicz-Szczytt 1997). R_0 genotypes derived from the same embryo should be theoretically identical according to their genome and their phenotypic expression without somaclonal variation (Niemirowicz-Szczytt 1997). High interline variability among DH lines originating from several clones among cabbage genotypes was also recorded by Dore & Boulidard (1988) whereas other lines were equivalent to pure lines from pedigree selection. Obtained in this paper results suggests that somaclonal variation among head cabbage plants of R_0 and R_1 generations may significantly decreased the value of cabbage androgenic genotypes from Jaguar F_1 hybrid. Somaclonal variation among head cabbage androgenic genotypes may have influence on the evaluation of internal homozygosity by the use of molecular markers. Androgenic lines of R_1 generation with high level of somaclonal variation might have been accessed as heterozygotes obtained from somatic tissue by mistake (Kamiński *et al.* 2003). Presence of somaclonal variation among androgenic cabbage plants required a special approach to eliminate genotypes with the lack of internal uniformity in respect of their utility for the breeding purposes. Strong and attentive selection of internally uniform genotypes of R_1 generation for desired commercial traits will effectively lead to obtain more stable and uniform R_2 generation. For that reason, head cabbage cultivars with their biennial habit may require two or more additional years of testing of androgenic material in respect of their genetic stability.

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Table 1. Androgenic head cabbage plants from Jaguar F₁ hybrid in consecutive generations, Skierniewice, Poland

Year of evaluation	Generation	Phase of development	Number of embryos	Number of plants	Action
2004	R ₀	Vegetative	11	103	Greenhouse vegetation
2005	R ₀ /R ₁	Generative	8	39	Greenhouse propagation
2006	R ₁	Vegetative	8	39	Field evaluation
2007	R ₁ /R ₂	Generative	7	12/8	Greenhouse/Field cages propagation
2008	R ₂	Vegetative	7	12/6	Field evaluation

Table 2. Seed setting of R₁ generation obtained from head cabbage androgenic R₀ plants from Jaguar F₁ donor cultivar. Skierniewice 2005

Embryo code	R ₀ plant number	Open flower pollination	Number of R ₁ seeds/pod		Average for embryo
			Average for embryo	Bud pollination	
2,1	14	0,71		0,39	
2,1	16	0.80		1.79	
2,1	31	0.03		2.78	
2,1	33	0.20	0.43	6.00	3.27
2,2	4	0.00		2.17	
2,2	7	0.00		0.40	
2,2	14	0.18		0.71	
2,2	15	0.28		1.33	
2,2	16	1.03		1.69	
2,2	19	0.88		4.20	
2,2	27	0.00	0.34	4.50	2.14
2,3	5	1.08		1.80	
2,3	6	1.25		1.92	
2,3	7	0.36		2.94	
2,3	8	0.00		1.50	
2,3	10	0.76		0.90	
2,3	12	0.41		1.65	
2,3	14	1.50		0.10	
2,3	17	0.01	0.67	1.69	1.56
4,2	4	0.00		5.17	
4,2	14	3.00		4.94	
4,2	15	0.85		6.15	
4,2	16	0.96		2.33	
4,2	17	0.88		2.40	
4,2	18	0.88	1.09	3.00	4.00
4,5	25	0.00	0.00	1.21	1.21
6,2	3	1.15		0.47	
6,2	7	1.08	1.11	1.89	1.18
6,5	1	0.00		0.90	
6,5	2	0.00		3.00	
6,5	6	0.17		0.78	
6,5	7	0.07		3.42	
6,5	8	0.17		2.63	
6,5	18	0.73		0.80	
6,5	19	0.22		0.63	
6,5	20	0.35		3.45	
6,5	21	0.27		4.75	
6,5	32	0.27	0.22	5.56	2.59
13,1	1	0.98	0.98	2.50	2.50
Average for all genotypes		0.53		2.42	

Table 3. Seed setting of R_2 generation obtained from head cabbage androgenic R_1 genotypes from Jaguar F_1 donor cultivar. Skierniewice 2007

R_1 line	Number of seeds/pod from self pollination in the greenhouse		Mass of seeds/plant (g) from sib pollination in the field-cage
	Open pollination	Bud pollination	
2,1/31	0,20	3,14	0.0
2,2/4	1.00	2.00	0.5
2,2/27	0.17	0.83	0.0
2,2/7	1.25	0.65	-
2,2/15	1.03	0.27	-
2,3/14	0.52	1.17	0.2
2,3/8	0.70	0.36	0.3
2,3/17	0.13	0.39	-
4,2/14	0.64	2.11	2.6
6,2/7	1.02	0.50	4.2
6,5/7	0.05	0.79	0.1
13,1/1	0.20	2.00	-
Average	0.54	1.18	

Table 4. Morphological characters of androgenic R₁ genotypes and Jaguar F₁ donor cultivar, Skierniewice 2006

Genotypes	Mass of head	Head shape (length/width) coefficient	Internal stump (length / head height) coefficient	Intraline uniformity	Period of vegetation
4,2/17	1,47 df	1,18 k	0,507 bg	3	95
2,2/7	1,63 cf	1,07 fk	0,487 bg	1	70
4,2/14	1,63 cf	1,18 k	0,487 bg	2	95
6,2/7	1,69 cf	0,93 af	0,453 af	1	95
4,2/4	1,71 cf	1,2 k	0,467 af	3	95
2,3/5	1,72 cf	1,03 ej	0,523 dg	2	75
6,2/3	1,75 cf	0,98 dh	0,530 eg	1	95
2,3/14	1,77 bf	1,03 ej	0,493 bg	1	70
2,2/4	1,77 bf	1,14 1,14	0,447 af	1	70
2,1/14	1,77 bf	1,11 hk	0,520 cg	1	80
2,1/16	1,81 bf	0,97 ch	0,557 fg	3	80
4,2/16	1,83 bf	1,18 k	0,443 af	3	95
4,2/18	1,83 bf	1,13 ik	0,513 bg	3	95
2,2/19	1,83 bf	1,09 gk	0,407 ac	1	70
13,1/1	1,85 be	1,00 ei	0,480 bg	1	90
6,5/1	1,86 be	0,83 ab	0,500 bg	1	90
6,5/32	1,87 be	0,89 ae	0,477 bg	2	90
2,3/6	1,88 be	1,06 fk	0,453 af	2	75
6,5/7	1,88 be	0,95 bg	0,510 bg	3	90
2,3/12	1,91 be	1,1 hk	0,400 ab	3	75
2,3/8	1,92 be	1,07 fk	0,510 bg	2	80
6,5/20	1,98 be	0,87 ad	0,523 dg	2	90
6,5/19	1,99 be	0,81 a	0,580 g	2	90
2,3/17	1,99 be	1,08 fk	0,490 bg	1	80
2,3/7	2,00 be	1,08 fk	0,477 bg	1	75
6,5/18	2,01 be	1,00 ei	0,467 bf	3	90
6,5/8	2,02 be	0,83 ab	0,473 bg	1	90
2,3/10	2,03 bd	1,02 ej	0,517 cg	3	75
2,2/27	2,05 bd	1,11 hk	0,450 af	1	75
2,2/14	2,06 bd	1,1 hk	0,470 bg	1	70
4,2/15	2,07 bd	1,02 ej	0,480 bg	3	95
6,5/6	2,07 bd	0,90 ae	0,500 bg	1	90
2,1/33	2,07 bd	0,98 dh	0,501 bg	3	80
6,5/21	2,11 bd	0,85 ac	0,527 eg	2	90
6,5/2	2,16 bd	0,85 ac	0,367 a	3	90
2,1/31	2,17 bd	1,03 ej	0,490 bg	2	80
2,2/15	2,18 bd	1,06 fk	0,417 ae	1	80
2,2/16	2,21 bc	1,02 ej	0,473 bg	1	80
4,5/25	2,47 b	1,17 jk	0,430 ae	1	95
Jaguar F ₁	3,18 a	1,00 ei	0,440 ae	1	95

Means followed by the same letter are not significantly different at $\alpha=0,05$

Intraline uniformity: 1 – lines uniform, 2 – lack of uniformity according to one trait, 3 – lack of uniformity according to more than one trait

Table 5. Morphological characters of androgenic R₂ genotypes and Jaguar F₁ donor cultivar. Skierniewice 2008

Genotype	Mass of head	Head shape (length/width) coefficient	Internal stump length / head height coefficient	Intraline uniformity	Period of vegetation
R ₂ genotypes from self pollination					
2,1/31	3,58 a	1,097 ac	0,46 ac	1	80
2,2/7	2,83 d	1,097 ac	0,47 ac	1	80
2,2/15	3,2 ac	1,12 bc	0,43 a	1	80
2,2/27	2,46 de	1,13 bc	0,43 a	1	80
2,2/4	2,46 df	1,12 bc	0,46 ab	1	80
2,3/8	1,93 gh	1,093 ac	0,49 ad	1	80
2,3/14	2,82 bd	1,083 ac	0,48 ac	1	80
2,3/17	2,83 bd	1,12 bc	0,49 ad	1	80
4,2/14	2,04 fh	1,293 d	0,43 a	1	95
6,2/7	1,78 h	1,100 ac	0,54 bd	2	110
6,5/7	2,70 d	0,993 a	0,56 d	1	110
13,1/1	2,4 df	1,060 ac	0,48 ad	1	110
R ₂ genotypes from sib pollination					
2,2/4 i	3,48 a	1,12 bc	0,43 a	1	80
2,3/8 i	1,39 i	1,177 c	0,44 a	1	80
2,3/14 i	2,60 de	1,050 ab	0,46 ab	1	80
4,2/14 i	2,54 de	1,330 d	0,48 ad	1	95
6,2/7 i	2,83 bd	1,093 ac	0,50 ad	1	110
6,5/7 i	2,21 eg	1,020 ab	0,55 cd	1	100
Jaguar F ₁	3,22ac	1,017 ab	0,45 a	1	100

Means followed by the same letter are not significantly different at $\alpha=0,05$

Intraline uniformity: 1 – lines uniform, 2 – lack of uniformity according to one trait, 3 – lack of uniformity according to more than one trait