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FIELD PERFORMACE OF 'SENGA SENGANA' STRAWBERRY PLANTS (*Fragaria* × *ananassa* Duch.) OBTAINED BY RUNNERS AND *IN VITRO* THROUGH AXILLARY AND ADVENTITIOUS SHOOTS

Wojciech Litwińczuk
Department of Plant Production, University of Rzeszów, Poland

ABSTRACT

Strawberry plants of 'Senga Sengana' *cv.* obtained *in vitro* from axillary (K-V0) and adventitious (P-V0) shoots were compared with their runner progeny (K-V1 and P-V1, respectively) and with standard runner (S, control) plants under field conditions. No differences were found in leaf shape and colour. In the planting year, *in vitro* obtained plants, both 'K-V0' and 'P-V0', developed significantly more crowns and runners while compared to other groups. Such differences, especially in runners' number were not observed in the next two years. Flowering behaviour was appreciably influenced by propagation method. In the planting year, all *in vitro* propagated plants and about 80% their runner progeny flowered contrary to control (the only 3% plants). Every year 'V0' plants developed significantly more inflorescences than other studied groups. Plants obtained *in vitro* produced bigger fruits and higher yield than other groups in the first two years. However, a reduction of berry yield for 'V0' plants in contrast with control was observed in third year only. Similarly, quality of fruits collected from 'V0' was improved in the first two years after planting and worsen in the last one while compared to control and 'V1' plants. Clustering analysis revealed two separated groups of plants: 'S' – 'P-V1' – 'K-V1' and 'K-V0' – 'P-V0', respectively.

Key words: field performance, micropropagation, strawberry, axillary shoots, adventitious shoots, runners, culture *in vitro*

INTRODUCTION

The method of micropropagation of strawberry was elaborated about thirty years ago Boxus [2]. However, there are still some problems with it. Adventitious shoots occur spontaneously and are common in strawberry cultures *in vitro* [2, 4, 9, 10, 11, 28]. Adventitious cultures of many species are suspected to be the main source of somaclonal variation [5]. Although many successful studies were carried out to improve adventitious buds initiation from callus [17, 24, 25, 30, 38, 39], leaves [28, 29, 31, 39], leaf petioles or stipules [4, 9, 10, 11, 12, 22, 25, 28] and roots [30] of strawberry, the adverse method of adventitious shoots' suppression without retardation of axillary shoots' growth is still not elaborated. On the other hand the micropropagation of strawberry by adventitious shoots should be more effective, easier and cheaper than by auxillary ones as the rate of multiplication of such shoots is often much higher [9, 10]. Numerous studies on field performance of micropropagated and standard (runner) strawberry plants were carried out. However, there exists little knowledge of the field behaviour of strawberries obtained *in vitro* from adventitious shoots. Swartz and Lindstrom [34], Marcotrigiano et al. [20], Boxus [2], Jemmali et al. [9] concluded that plants of adventitious origin produce more stolons and flowers. It was confirmed by Janečkova et al. [8] in the case of 'Senga Sengana' somaclones obtained from leaf discs. Some authors reported that hyperflowering may be reduced even by single propagation of TC plants by runners [1, 15, 19, 34]. Micropropagated strawberry plantlets readily develop runners even at the end of adaptation stage especially under long-day photoperiod. Thus, standard method of strawberry propagation (by runners) may be applied very early, in the glasshouse. It should be interesting to reveal whether such plants are more similar to plants obtained by runners in the field nurseries or to those obtained *in vitro*. The aim of the study was to find out whether growth and fruiting habit of strawberry plants are affected by propagation method.

MATERIALS AND METHODS

The experiment was carried out in years 1999-2001 on five groups of 'Senga Sengana' strawberry plants (*Fragaria* × *ananassa* Duch.) obtained by runners and by micropropagation (tab. 1). Cultures *in vitro* were established in the Research Institute of Floriculture and Pomology in Skierniewice. Then they were grown in Department of Plant Production of Cracow Agricultural University (currently Department of Plant Production of Rzeszów University). The medium recommended by Boxus [2] supplemented with 6-benzylaminopurine (BA, 0.5 mg dm^{-3}), gibberellic acid (GA_3, 0.1 mg dm^{-3}), indole-3-butyric acid (IBA, 0.1 mg dm^{-3}), glucose (40.0 g dm^{-3}) and Bacto-Difco agar (6.4 g dm^{-3}) was applied. Two kinds of explants were used: rosettes and leaf petioles (with stipules) to obtain axillary (K) and adventitious (P) shoots. Afterwards shoots were multiplied and subsequently rooted *in vitro* on Boxus medium without BA and GA_3. Obtained plants (V0) were transplanted to mixture of peat, perlite and Groadan (6:2:2 v/v, pH = 5.5) fertilised with 'Peters Professional Plant Starter^{TM}' in the end of October 1998. They have been grown for 3 weeks at high air humidity in 16h/8h day/night photoperiod under sodium light at 64.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD and 21±3°C temperature. In mid December 1998 plants were replanted to 0.2 dm^{-3} pots. During adaptation plants developed runners which were used to obtain next generation (V1). Control (S) strawberry plants came from the Fruit Experimental Station Research Institute of Floriculture and Pomology Brzezna Ltd. The were digged up from field nursery in the spring. Both 'V1' and 'S' plants were also transferred to 0.2 dm^{-3} pots filled with the same medium as that for 'V0' plants in mid April 1999. All plant were sprayed every 2 weeks with solution of 'Florovit^{TM}' fertilizer (1%) and fungicides, like: Previcure^{TM} (0.15%), Topsin M^{TM} (0.1%), Rovral^{TM} (0.2%) or Euparen^{TM} (0.2%). Before planting to the field plants have been hardened for 1 month. A randomised block design (5 blocks × 6 plants in block for every of 5 propagation methods) was established in May 1999. Plants were placed at 0.4 m × 0.35 m distance on 1 m wide raised bed covered with black 'Neotex^{TM}' fabric in loess brown soil (pH 5.6) fertilised with 75 kg N ha^{-1}, 100 kg K_2O ha^{-1} and 50 kg P_2O_5 ha^{-1}. Plants were watered immediately after planting. Thereafter they were not irrigated. Plant were sprayed about 4 times per year with solution of fungicides: Topsin M^{TM} (0.1%) and Rovral^{TM} (0.2%) or Euparen^{TM} (0.2%) and fertilizers: 'Florovit^{TM}' (1%) or 'Nowokont^{TM}' (1%). Other pesticides and fertilizers were not applied. Every year in autumn all runners were removed. Aditionally, in 2000, all leaves were cut off after fruit harvest.

Table 1. Short description of examined groups of strawberry plants

Symbol	Method of propagation
S (control)	Fourth runner generation of micropropagated mother plants
K-V0	Plants propagated *in vitro* by axillary shoots
P-V0	Plants propagated *in vitro* by adventitious shoots which occurred spontaneously on leaf stipules
K-V1	First generation of 'K-V0' plants obtained from runners which developed at the end of adaptation stage of micropropagated plants
P-V1	First generation of 'P-V0' plants obtained from runners which developed at the end of adaptation stage of micropropagated plants

Every year the following traits were recorded: angle of terminal leaflet base (on 3 typical leaves), number of crowns, runners, inflorescences per plant, number of flowers (on 3 typical inflorescences). In 1999 colours of leaf blades and stipules were evaluated and berry size was scored (1 – φ < 1 cm; 5 – φ > 4 cm). During the period 2000-2001 the size and weight of 3 ripe fruits per plant was recorded. This was done in 12 pickings (6/year) every 3 days. The total number of ripe fruits per plant was also noted in every picking. Collected data were submitted to ANOVA, LSD mean separation test at p = 0.05 significance level and cluster analysis according to Ward's method using Statgraphics 4.2 and Statistica 5.1 computer software.

RESULTS

No differences among examined plants were found in colour of leaves and stipules. The shape of terminal leaflet as well as the angle of its base remained unchanged also (tab. 2).

Table 2. Effect of propagation method on angle of terminal leaflet base

Propagation method	Year					
	1999		2000		2001	
	Mean, °	CV[a], %	Mean, °	CV, %	Mean, °	CV, %
S	118 a	6.4	111 a	12.3	107 a	9.6
K-V1	120 a	7.4	115 a	7.6	110 a	9.3
P-V1	119 a	7.6	110 a	10.9	107 a	8.8
K-V0	120 a	8.4	110 a	8.6	110 a	8.3
P-V0	118 a	7.8	114 a	9.8	108 a	8.1
$LSD_{0.05}$[b]	4.6	-	5.9	-	5.6	-
SL[c]	ns	-	ns	-	ns	-

[a] coefficient of variation,
[b] least significant difference,
[c] level of significance

In the planting year, *in vitro* obtained plants, both 'K-V0' and 'P-V0', developed significantly more crowns while compared to other groups (tab. 3). In the second year such differences were significant among 'V0' plants and their progeny (V1) whereas in the third year the only 'P-V0' plants produced significantly more crowns. The variation of all examined groups of plants was similar (tab. 3).

Table 3. Effect of propagation method on number of crowns per plant

Propagation method	Year					
	1999		2000		2001	
	Mean, pcs	CV, %	Mean, pcs	CV, %	Mean, pcs	CV, %
S	1.8 a[d)]	45.0	4.1 b	32.2	4.9 a	23.7
K-V1	1.5 a	36.7	3.1 a	37.7	5.0 a	28.2
P-V1	1.8 a	51.1	3.3 a	33.3	4.8 a	25.8
K-V0	3.4 b	44.4	4.3 b	28.8	5.2 a	31.7
P-V0	3.3 b	42.7	4.3 b	32.3	6.0 b	19.5
$LSD_{0.05}$	0.77	-	0.64	-	0.64	-
SL	***	-	**	-	**	-

[d)] different letters indicate significant differences for $p < 0.05$

Differences among studied groups in plant runnering were visible in the first year only (tab. 4). Micropropagated plants developed significantly more stolons and were more uniform than their progeny (V1) and control plants (S). Such differences were not observed in the next two years (tab. 4).

Plant runnering was most intense in second year whereas was strongly inhibited in third year after planting. The behaviour of all tested groups was similar (tab. 4).

Table 4. Effect of propagation method on plant runnering (number of stolons per plant)

Propagation method	Year					
	1999		2000		2001	
	Mean, pcs	CV, %	Mean, pcs	CV, %	Mean, pcs	CV, %
S	2.1 b	96.2	16.2 a	42.3	3.5 a	39.1
K-V1	0.3 a	167.0	16.3 a	35.6	4.2 a	28.6
P-V1	1.3 ab	127.0	16.8 a	33.2	3.6 a	39.7
K-V0	4.6 c	50.2	15.8 a	36.8	3.8 a	41.6
P-V0	3.9 c	63.3	14.1 a	38.3	4.0 a	32.5
$LSD_{0.05}$	0.99	-	3.04	-	0.72	-
SL	***	-	ns	-	ns	-

Flowering of plants was appreciably influenced by propagation method. In the planting year, all *in vitro* obtained plants and majority of their runner progeny (78% and 82% for 'K-V1' and 'P-V1', respectively) flowered, contrary to control (only 3% plants). The differences among 'V0', 'V1' and 'S' groups were significant. All examined plants bloomed in the next two years. Every year micropropagated plants formed significantly more inflorescences than other studied groups (tab. 5). The only in the first year the number of inflorescences determined for plants of adventitious origin 'P-V0' was higher than that for plants multiplied by axillary shoots 'K-V0'. The 'V0' plants were more uniform than their progeny (V1). The differences among plants propagated by runners ('V1' and 'S') were not found in 1999 and 2001. However, in 2000 the 'P-V1' plants developed more inflorescences than 'S' and 'K-V1' ones.

Table 5. Effect of propagation method on number of inflorescences per plant

Propagation method	Year					
	1999		2000		2001	
	Mean, pcs	CV, %	Mean, pcs	CV, %	Mean, pcs	CV, %
S	0.3 a	-[e)]	10.1 a	36.1	5.1 a	45.1
K-V1	0.8 a	102.9	10.6 a	64.3	4.7 a	53.2
P-V1	1.0 a	89.1	15.6 b	43.9	5.7 ab	43.9
K-V0	4.4 b	49.1	27.5 c	33.4	7.8 c	41.9
P-V0	5.5 c	49.2	26.5 c	37.7	6.9 bc	38.4
$LSD_{0.05}$	0.83	-	3.92	-	1.36	-
SL	***	-	***	-	***	-

[e)] Only one plant bloomed

Some differences among studied plants were found while the number of flowers in inflorescence was considered. However, they were not year-stable. The control plants formed less complex inflorescences in the first two years than other groups (tab. 6). Such difference was not proved in 2001. At that year the 'P-V1' plants developed less flowers in inflorescence. Other differences were not statistically proved (tab. 6). The variation of all studied groups was similar.

Table 6. Effect of propagation method on number of flowers per inflorescence

Propagation method	Year					
	1999		2000		2001	
	Mean, pcs	CV, %	Mean, pcs	CV, %	Mean, pcs	CV, %
S	4.0 -[f)]	-	5.6 a	37.5	7.0 bc	28.0
K-V1	5.2 ± 0.81 a	38.6	6.6 b	35.2	7.4 c	27.6
P-V1	6.7 ± 0.68 a	34.5	6.0 ab	39.2	6.4 a	33.3
K-V0	5.5 ± 0.59 a	28.5	6.6 b	28.8	6.9 abc	29.1
P-V0	5.7 ± 0.59 a	26.5	6.5 b	29.4	6.8 ab	27.5
$LSD_{0.05}$	-[g)]	-	0.62	-	0.6	-
SL	ns	-	**	-	*	-

[f)] 'S' group was excluded from analysis as the only one plant bloomed.
[g)] Different size of LSD because of various number of flowered plants.

The plants obtained *in vitro* (V0) produced significantly more fruits than other groups both in 2000 and 2001 (tab. 7). It was observed also that the 'P-V1' plants gave more berries than 'S' and 'K-V1' ones in 2000. All studied groups of plants bore more fruits in 2000 than in 2001. However, stronger reduction in fruit number was noted in case of 'V0' plants (-69% and-65% for 'P-V0' and 'K-V0', respectively) than for their progeny and control (-61%, -53%, -39% for 'P-V1', 'K-V1' and 'S', respectively). The 'V0' plants were more uniform in number of harvested fruits than their progeny in 2000. The variation of all studied groups in 2001 was similar (tab. 7).

Table 7. Effect of propagation method on number of fruits collected from plant

Propagation method	Year			
	2000		2001	
	Mean, pcs	CV, %	Mean, pcs	CV, %
S	29.0 a	36.7	17.6 ab	44.5
K-V1	31.0 a	60.5	14.6 a	51.2
P-V1	43.1 b	42.3	16.8 ab	48.6
K-V0	65.6 c	29.1	23.2 c	46.6
P-V0	67.5 c	35.8	20.9 bc	47.2
$LSD_{0.05}$	9.48	-	4.64	-
SL	***	-	*	-

Visible differences in plant yielding were observed in the first two years while the 'V0' groups yielded significantly better than other groups (tab. 8). (The fruit yield for 1999 was not determined. However, it should be much higher for 'V0' plants as they developed significantly more inflorescences than other groups). In general the highest yield of fruits was obtained in 2000. In the following year a drastic reduction in plant yielding was observed in the case of 'V0' plants (-58% and -48% for 'P-V0' and 'K-V0', respectively). It was not so strong for their progeny (-42% and -19% for 'P-V1' and 'K-V1', respectively) and was not noted for the control plants where yield of fruits even increased (+12%). Despite of it, the differences among studied groups in plant yielding were not confirmed in 2001 (tab. 8). The 'V0' plants were more uniform in fruit harvest than their progeny and control in 2000. The variation of all studied groups in 2001 was similar (tab. 8).

Table 8. Effect of propagation method on yield of fruits collected from plant

Propagation method	Year			
	2000		2001	
	Mean, g	CV, %	Mean, g	CV, %
S	145 a	56.5	162 a	45.3
K-V1	175 a	70.5	142 a	47.7
P-V1	253 b	50.0	146 a	47.3
K-V0	361 c	34.3	188 a	49.0
P-V0	395 c	41.9	166 a	43.0
$LSD_{0.05}$	64.4	-	46.4	-
SL	***	-	ns	-

The fruit size and weight were affected by propagation method and year of harvest (tab. 9-12). In general, fruits collected from 'V0' plants were slightly bigger in comparison to those gathered from 'V1' plants in first two years contrary to control berries which were significantly smaller in 2000. Such phenomena had diametrically changed in the last year of harvest while berries picked from 'V0' plants were smaller in contrast to fruits collected from other plants, both 'S' and 'V1' (tab. 10-12).

Table 9. Effect of propagation method on berry size in the year of planting

Propagation method	Mean (1-5)	CV, %
S	-[h)]	-
K-V1	2.6 ± 0.32 ab	20.4
P-V1	2.0 ± 0.29 a	39.0
K-V0	3.2 ± 0.28 b	40.3
P-V0	3.2 ± 0.28 b	39.1
$LSD_{0.05}$	-[i)]	-
SL	***	-

[h,i)] explanations, see table 6

Table 10. Effect of propagation method on mean berry weight

Propagation method	Year			
	2000		2001	
	Mean, g	CV, %	Mean, g	CV, %
S	6.3 a	22.5	9.1 bc	14.3
K-V1	6.4 ab	18.8	9.1 bc	12.4
P-V1	6.9 bc	17.4	9.1 c	18.0
K-V0	7.0 bc	13.0	8.2 a	13.4
P-V0	7.1 c	14.8	8.4 ab	14.8
$LSD_{0.05}$	0.60	-	0.68	-
SL	**	-	*	-

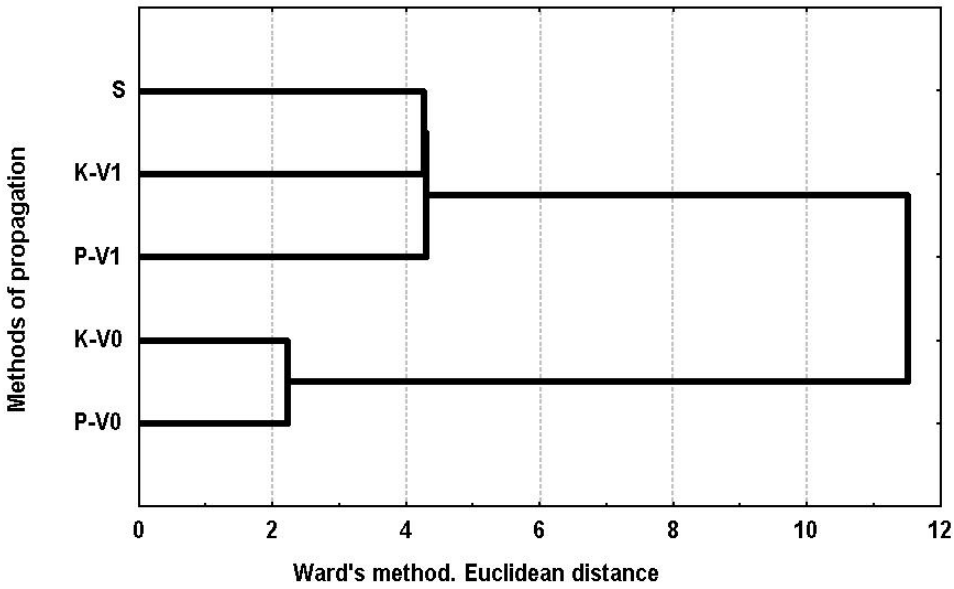
Table 11. Effect of propagation method on mean berry width

Propagation method	Year			
	2000		2001	
	Mean (cm)	CV (%)	Mean (cm)	CV (%)
S	2.4 a	7.9	2.7 a	6.0
K-V1	2.4 ab	7.0	2.7 a	3.8
P-V1	2.5 b	6.8	2.7 a	7.1
K-V0	2.5 b	5.2	2.6 a	6.2
P-V0	2.5 b	6.3	2.6 a	5.0
$LSD_{0.05}$	0.09	-	0.08	-
SL	*	-	ns	-

Table 12. Effect of propagation method on mean berry height

Propagation method	Year			
	2000		2001	
	Mean, cm	CV, %	Mean, cm	CV, %
S	2.2 a	9.6	2.4 ab	5.4
K-V1	2.3 b	7.5	2.6 c	5.0
P-V1	2.3 b	6.9	2.5 b	14.3
K-V0	2.3 b	5.6	2.4 a	7.2
P-V0	2.3 b	6.1	2.4 ab	6.7
$LSD_{0.05}$	0.08	-	0.10	-
SL	**	-	*	-

Figure 1. Similarity of examined groups of strawberry plants



Clustering analysis, based on traits for which significant differences among studied plants were found, revealed two separated groups of plants, one propagated by runners: 'S' – 'K-V1' – 'P-V1' and second obtained directly *in vitro*: 'K-V0' – 'P-V0' (fig. 1).

DISCUSSION

Numerous studies have been published regarding field behaviour of micropropagated strawberry. Such plants more or less often exhibit characteristics, like: dwarfism, chlorosis or white striking of leaves, stem fasciations, intensified vigour, hyperrunnering and abnormal flowering (hyperflowering) accompanied by increased production of smaller fruits. Some of them were confirmed in the presented study on micropropagated 'Senga Sengana' strawberry plants and their progeny. Any alterations were not found in leaf colour and shape. Distinct differences in number of crowns and stolons among micropropagated and control plants were found in the first year only. They could be explained both by well-known increased vigour of TC plants as well as by diminished stress of 'V0' plants which were set into soil with undamaged roots contrary to 'S' plants. The most visible and stable aftermath of micropropagation were: readiness of 'V0' plants to bloom in the year of planting and hyperflowering caused by increased number of inflorescences. It should be emphasised that in the first two years 'V0' plants produced higher yield and bigger fruits in comparison to conventionally propagated plants. Similar results (at least the same yield and fruit quality of 'V0' and control plants) were obtained by [3, 16, 18, 23]. Such phenomena would be beneficial to the fruit growers. Nevertheless, it should be also underlined that more authors reported deterioration of fruit quality in the case of micropropagated plants [7, 13, 14, 15, 21, 31, 33, 36, 37]. In the presented study a sharp decrease in yielding and relative smaller fruits of 'V0' plants were observed in the third year after planting. Such phenomena could be explained by: exhausting of 'V0' plants by abundant yielding in the previous year, depletion of nutrients in the soil caused by intensive growth and cropping, stronger plant competition, *etc*. Similar conclusion was drawn by Nehra et al. [23] who suggested that differences in fruit production and quality may have been due to overcrowding of 'V0' plants. Szczygieł and Borkowska [35] found different reaction of micropropagated and control plants on various levels of fertilisation. Thus it is very probably that requirements of micropropagated plants are quite different from those of traditionally propagated plants. Therefore a specific agro-technique for 'V0' strawberry plants should be elaborated.

Presented study revealed very close similarity 'V0' plants obtained from axillary (K-V0) and adventitious (P-V0) shoots. In general significant differences between such plants (except for number of crowns in 2001 and number of inflorescences in 1999) were not found. More abundant flowering and often bigger fruit size of 'Senga Sengana' somaclones obtained from leaf discs against meristem plants was noted by Janečkova et al. [8]. They also found differences among studied somaclones. Unfortunately they published only 1-year results and there is no information whether such alterations were stable in following years. Contrary to report of Janečkova et al. [8] in presented study plants of adventitious origin obtained from leaf stipules were as uniform as plants developed from axillary shoots. May be the reason is the different type of explant used (leaf discs *versus* leaf stipules). Therefore, adventitious shoots which readily and spontaneously occur in strawberry cultures *in vitro* are not the only reason of distinctness of 'V0' plants. However, it is also possible that changes which lead to hyperflovering take place during culture initiation and stabilisation and have not constant but one-step character.

Some authors revealed that hyperflowering was appreciable reduced in first generation of micropropagated plants obtained traditionally, by runners [1, 15, 19, 34]. Such observation was confirmed in the presented study. Strawberry plants (V1) obtained by runners from very young micropropagated plants (V0) at the end of adaptation stage bore stronger resemblance to control (S) plants than to mother ones (V0). It creates opportunity to join micropropagation with 'soiless' method of strawberry propagation [6, 26, 27] to obtain nursery plantlets of high quality.

CONCLUSIONS

1. The most visible and year-stable aftermath of micropropagation was hyperflowering caused by increased number of inflorescences. Such phenomena was not correlated with deterioration of fruit quality.
2. Very close similarity 'V0' plants obtained *in vitro* from axillary (K-V0) and adventitious (P-V0) shoots was revealed.
3. Strawberry plants (V1) obtained by runners from very young micropropagated plants (V0) at the end of adaptation stage bore stronger resemblance to control (S) plants than to mother ones (V0).

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Wojciech Litwińczuk
Department of Plant Production, University of Rzeszów
2 Ćwiklińskiej Street, 35-601 Rzeszów, Poland
tel. (+48 17) 857 42 55 ext. 341 or 219
e-mail: wlitw@univ.rzeszow.pl
