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INFLUENCE OF FOLIAR ANTIBROADLEAVED HERBICIDES ON COTTON SEED GERMINATION (*GOSSYPIUM HIRSUTUM* L.)

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ABSTRACT

The trial was carried during period 2013-2015 with twelve Bulgarian cotton cultivars - Chirpan-539, Helius, Trakia, Viki, Filipopolis, IPK-Veno, Boyana, Avangard, Natalia, Darmy Dorina and Nelina (*Gossypium hirsutum* L.). Herbicides Bazagran 480 SL (bentazone), Pulsar 40 (imazamox) and Express 50 SX (tribenuron-methyl), applied during budding stage of cotton, were investigated. For the first time in the world, cotton cultivars resistant to herbicides Basagran 480 SL, Pulsar 40 and Express 50 SX were identified. Herbicide Bazagran 480 SL had the highest phytotoxicity on seed germination in cultivars Chirpan-539. Herbicide Pulsar 40 had the highest phytotoxicity on seed germination in cultivar Filipopolis. Herbicide Express 50 SX had the highest phytotoxicity on seed germination in cultivar Chirpan-539. From the viewpoint of technology for cotton growing, in terms of its impact on germination of cotton seeds, technologically the most suitable to foliar treatment with herbicide Bazagran 480 SL are cultivars Helius, Trakia, Viki, Filipopolis, IPK-Veno, Boyana, Avangard, Natalia, Darmy Dorina and Nelina. For the first time in the world we established that in the vegetative treatment with herbicides, technologically the most suitable to foliar treatment with herbicide Pulsar 40 are cultivars Helius, Trakia, IPK-Veno, Boyana. Technologically the most suitable to foliar treatment with herbicide Express 50 SX are cultivars Filipopolis, IPK-Veno, Avangard, Natalia and Dorina. These variants combine high levels of the seed germination and high stability of this index during the years.

Key words: cotton, herbicides, foliar treatment, cultivars, seed germination.

INTRODUCTION

Cotton is a crop characterized by long vegetation period and a poor competitive ability to weeds. Because of this, it is highly sensitive to weed spread from the earliest stages of its development.

Problems with primary weed spread in cotton are solved to a considerable extent (Hakoomat, 2005; Chachalis and Galanis, 2007; Kahramanoglu and Uygur, 2010). Chemical control is the most effective method of weed control in cotton (Saldzhiev et al., 2008; Delchev, 2018). The issue of secondary weed spread of annual and perennial graminaceous weeds during cotton vegetation is also solved to a great extent by using antigraminaceous herbicides (Boz, 2000; Bukun, 2005; Cardoso, 2011; Jiang, 2012).

Data on herbicides for efficient control of secondary emerging annual and perennial broadleaf weeds in conventional cotton growing technology are rather scarce even on a global scale. Effective herbicides for their control in cotton are still being sought. In the application of vegetative antibroadleaved herbicides in conventional technology, there are often manifestations of phytotoxicity (Barakova and Delchev, 2016; Barakova, 2017).

Information on glyphosate-tolerant and glufosinate-tolerant cotton cultivars was presented (Gaylon et al., 2015; Spielman et al., 2015). In them control of all weeds – graminaceous and broadleaf, annual and perennial is completely solved by the use of total herbicides based on glyphosate (Roundup Ready technology) or glufosinate (Liberty Link technology). These two technologies are widely used in major cotton-producing countries. However, these cultivars are GMOs and are banned within the territory of the European Union. This

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makes the present study particularly relevant for all cotton producing countries within the European Union.

Often, when applying herbicides during crop vegetation, phytotoxicity affects the growth and development, yield and quality of the fiber (Vargas and Wright, 1994; Gao, 2005; Ashok et al., 2006, Stoychev et al., 2010). Effective and selective vegetative herbicides for cotton are still being sought. There is insufficient research on their impact on the sowing characteristics of cotton seeds. The scientific literature does not have enough information on these issues.

The purpose of this study was to investigate the effect of vegetative treatment with the herbicides Basagran 480 SL, Pulsar 40 and Express 50 SX on the laboratory seed germination of different Bulgarian cotton (*Gossypium hirsutum* L.) cultivars.

MATERIAL AND METHODS

During the period 2013-2015 a field experiment was conducted in non-irrigated conditions, on pellic vertisol soil type. The experiment was carried out with twelve Bulgarian cotton cultivars - Chirpan-539, Helius, Trakia, Vicky, Philipopolis, IPK-Veno, Boyana, Avangard, Natalia, Darmy, Dorina and Nelina belonging to *Gossypium hirsutum* L. species. These cultivars are not genetically modified. They were created by interspecies hybridization and experimental mutagenesis.

Herbicides Basagran 480 SL (bentazone), Pulsar 40 (imazamox) and Express 50 SX (tribenuron-methyl) were studied. They were applied at the bud formation stage of cotton. Spraying was made with a hand back sprayer with a work solution of 300 l/ha. The herbicides were applied against the background of the herbicidal combination Dual gold 960 EC (S-metolachlor) + Goal 2 E (oxyfluorfen), applied after sowing pre-emergence to control primary weeding of cotton. The experiment variants are given in Table 1.

Cotton seed germination was determined for 100 seeds per each variant (25 seeds per 1 replication) of twelve varieties of cotton. The seeds were taken from cotton plants treated during the vegetation with the respective herbicides. Seed germination was reported on day 7.

Statistical evaluation to rate the representative and reliable effect of the studied parameters was applied through dispersion analysis and Fischer's parametric criterion F (Shanin, 1977; Barov, 1982). In the variance analysis the ANOVA123 software was used for calculation (Lidanski, 1988).

The selectivity of herbicides was established through their effect on cotton yield and the following variances were calculated:

Shukla, (1972) stability variance (σ_i^2)

$$Sh-\sigma_i^2=\left[1/(e-1)(t-1)(t-2)\right]\times\left[t(t-1)\sum_{j=1}^{s}(u_{ij}-\bar{u}_i)^2-\sum_{i=1}^{t}\sum_{j=1}^{s}(u_{ij}-\bar{u}_i)^2\right]$$

where:

$u_{ij} = X_{ij} - \bar{X}_{.j}$

$X_{ij} =$ observed trait value of i^{th} cultivar in j^{th} environment;

$\bar{X}_{.j} =$ mean of all cultivars in j^{th} environment;

$$\bar{u}_{i.} = \sum_{j=1}^{m} u_{ij}/e$$

$e =$ number of environments;

$t =$ number of cultivars.

In this study, calculation of adjusted stability variance ($Sh - S_i^2$) was necessary, because the heterogeneity term was significant ($p < 0.01$). The stability statistic $Sh\text{-}Si^2$ calculated following removal of heterogeneity due to environmental index ($Z_j = \bar{X}_{.j} - \bar{X}_{..}$) as a covariate from GE interaction variance, where $\bar{X}_{.j} =$ mean of all cultivars in j^{th} environment of all cultivars across all environments, using the following equation (Shukla 1972):

$$Sh-S_i^2=\left[t/(t-2)(e-2)\right]\times\left[s_i-\sum_{i=1}^{t}s_i/t(t-1)\right]$$

Table 1. Investigated variants

Herbicides						Cultivars (no GMOs)
After sowing, before emergence			During budding stage			
Herbicide	Active substance	Dose	Herbicide	Active substance	Dose	
Dual gold 960 EK + Goal 2 E	S-metolachlor + oxyfluorfen	1.2 l/ha + 1.5 l/ha	Basagran 480 SL	bentazone	1.5 l/ha	Chirpan-539
						Helius
						Trakia
						Vicky
						Philipopolis
						IPK-Veno
						Boyana,
						Avangard
						Natalia
						Darmy
						Dorina
						Nelina
			Pulsar 40	imazamox	1.2 l/ha	Chirpan-539
						Helius
						Trakia
						Vicky
						Philipopolis
						IPK-Veno
						Boyana,
						Avangard
						Natalia
						Darmy
						Dorina
						Nelina
			Express 50 SX	tribenuron-methyl	50 g/ha	Chirpan-539
						Helius
						Trakia
						Vicky
						Philipopolis
						IPK-Veno
						Boyana,
						Avangard
						Natalia
						Darmy
						Dorina
						Nelina

where:

$$s_i = \sum_{j=1}^{s}(u_{ij} - \bar{u}_{i.} - b_i Z_j)^2$$

$$b_i = {\sum_{j=1}^{s}[(u_{ij} - u_{i.})Z_j]} \Big/ {\sum_{j=1}^{s} Z_j^2}$$

Cultivar stability across multiple years and locations was also evaluated using the ecovalence (W_i) (Wricke 1962):

$$W_i^2 = \sum_{i=1}^{t}(X_{ij} - \bar{X}_{i.} - \bar{X}_{.j} + \bar{X}_{..})^2$$

Greatest stability is when

$$W = W_i^2 = 0 .$$

For cotton yields stability parameters were calculated. Stability variances (σ_i^2 and S_i^2) by Shukla (1972) and ecovalence W_i by Wricke (1962) show what portion of variation related to interaction of the treatments and years are accounted by the specific variant. Through the stability criterion (YS_i) of Kang (1993) the value of each variant was shown by simultaneously taking into account the parameter value and the stability of the variant. The value of that criterion is that by using non-parametric methods and statistical reliability of differences we obtain a combined valuation ranking variants in a descending order according to their economic value.

To calculate these parameters, the STABLE software of Louisiana State University Agricultural Center, Baton Rouge, USA (1993) was used. The following model was applied to assess the stability of various variants in their interaction with years:

$$X_{ij} = m + N_i + Y_j + NY_{ij} + L_{ij}$$

where:

X_{ij} – grain parameter (yield, mass) of the i^{-th} variant with j-th environment (year),
m – general mean;
N_i – effect of the i^{th} variant;
Y_j – effect of the j^{th} environment (year);
NY_{ij} – effect of interaction of the i^{th} variant with the j^{th} environment (year);
L_{ij} – error relating to the i^{th} variant in the j^{th} environment (year).

RESULTS AND DISCUSSION

Genetically improved cultivars and hybrids resistant to herbicides are widely used in many field crops in the world. These cultivars and hybrids are not GMOs and they are used within the territory of all European Union. In Bulgaria with their help are solved the problems of secondary weed infestation in field crops such as sunflower, maize, canola (Delchev, 2018).

Secondary weed infestation with annual and perennial broadleaf weeds is a huge problem for the cotton fields. To combat these weeds 3-4 hands hoeing with hoes are made. They are very heavy, labour intensive, and greatly increase the cost of cotton production. Until now, this has made conventional cotton production unprofitable and it could not compete with cheap GMO cotton produced in the major cotton-producing countries outside Europe.

We searched a significant number of studies to find cotton cultivars resistant to foliar-applied antibroadleaved herbicides. For the first time in the world, cotton cultivars resistant to herbicides Basagran 480 SL (bentazone), Pulsar 40 (imazamox) and Express 50 SX (tribenuron-methyl) were identified. These cultivars are Bulgarian and were created in the Field Crops Institute, Chirpan. They produce high and stable yields of raw cotton, cotton fiber and cotton seeds over the years (Barakova and Delchev, 2016; Barakova, 2017).

The use of the herbicides bentazone, imazamox and tribenuron-methyl in cotton provides complete control of late spring annual broadleaf weeds *Xanthium strumarium* L., *Amaranthus retroflexus* L., *Amaranthus albus* L., *Amaranthus blifoides* W., *Chenopodium album* L., *Solanum nigrum* L., *Datura stramonium* L., *Polygonum aviculare* L., *Abutilon teophrasti* Medic., *Portulaca oleracea* L., *Polygonum aviculare* L., *Hibiscum trionum* L., *Tribulus terrestris* L.

The herbicides imazamox and tribenuron-methyl also provide complete control of the perennial broadleaf weeds *Cirsium arvense* Scop. and *Convolvulus arvensis* L. (Barakova, 2017).

The obtained results are of great importance not only for Bulgaria and Romania, but also for other countries producing cotton in the European Union - Greece, Italy, Spain and Portugal, who cannot use genetically modified cotton cultivars. This requires a study of the quality indicators of the fibre and seeds of these genetically improved cultivars resistant to herbicides.

On average during the study period, the herbicide Basagran 480 SL applied during cotton vegetation (Table 2), showed the highest phytotoxicity on cotton seed germination of the Chirpan-539 cultivar.

The lowest value of this indicator as compared to the other cultivars was 64.2%. The weakest effect of the herbicide was seen on cultivar Natalia – 98.2%, followed by cultivars Helius and Boyana – 97.3%, which had the highest seed germination.

Table 2. Laboratory cotton seeds germination under influence of vegetation treatment with herbicides, % (2013 - 2015)

Herbicides	Cultivars	2013	2014	2015	Mean
Basagran	Chirpan-539	85.0	85.0	82.5	64.2
	Helius	97.0	97.5	97.5	97.3
	Trakia	92.0	85.0	92.5	89.8
	Vicky	92.0	90.0	92.5	91.5
	Philipopolis	95.0	100.0	95.0	96.7
	IPK-Veno	95.0	92.5	95.0	94.2
	Boyana.	97.0	97.5	97.5	97.3
	Avangard	92.0	87.5	92.5	90.7
	Natalia	97.0	97.5	100.0	98.2
	Darmy	97.0	97.5	97.5	97.3
	Dorina	95.0	95.0	95.0	95.0
	Nelina	97.0	90.0	97.5	94.8
Pulsar	Chirpan-539	92.0	82.5	92.5	89.0
	Helius	92.0	87.5	92.5	90.7
	Trakia	92.0	85.0	92.5	89.8
	Vicky	90.0	90.0	87.5	89.2
	Philipopolis	80.0	55.0	80.0	71.7
	IPK-Veno	97.0	77.5	97.5	90.7
	Boyana.	92.0	92.5	87.5	90.7
	Avangard	82.0	82.5	80.0	81.5
	Natalia	92.0	92.5	85.0	89.8
	Darmy	90.0	90.0	87.5	89.2
	Dorina	77.0	70.0	77.5	74.8
	Nelina	85.0	65.0	85.0	78.3
Express	Chirpan-539	80.0	80.0	80.0	80.0
	Helius	95.0	77.5	95.0	89.2
	Trakia	90.0	90.0	90.0	90.0
	Vicky	95.0	77.5	95.0	89.2
	Philipopolis	97.0	87.5	97.5	94.0
	IPK-Veno	97.0	80.0	97.5	91.5
	Boyana.	87.0	87.5	82.5	85.7
	Avangard	92.0	85.0	92.5	89.8
	Natalia	95.0	90.0	95.0	93.3
	Darmy	90.0	70.0	90.0	83.3
	Dorina	95.0	85.0	95.0	91.7
	Nelina	87.0	77.5	87.5	84.0

LSD, %:

F.A	$p\leq5\%=0.4$	$p\leq1\%=0.5$	$p\leq0.1\%=0.7$
F.B	$p\leq5\%=0.4$	$p\leq1\%=0.5$	$p\leq0.1\%=0.7$
F.C	$p\leq5\%=0.8$	$p\leq1\%=1.0$	$p\leq0.1\%=1.3$
AxB	$p\leq5\%=0.7$	$p\leq1\%=0.9$	$p\leq0.1\%=1.2$
AxC	$p\leq5\%=1.4$	$p\leq1\%=1.8$	$p\leq0.1\%=2.3$
BxC	$p\leq5\%=1.4$	$p\leq1\%=1.8$	$p\leq0.1\%=2.3$
AxBxC	$p\leq5\%=2.4$	$p\leq1\%=3.1$	$p\leq0.1\%=4.0$

The herbicide Pulsar 40 had the strongest phytotoxic effect on seed germination in seeds of Philipopolis cultivar – 71.7%. The cultivars Helius, IPK-Veno and Boyana had the highest values of the indicator compared to the other cultivars – 90.7%. These results show that the herbicide had the weakest effect on seed germination of these cultivars.

In the vegetative treatment with the herbicide Express 50 SX the highest phytotoxicity on the laboratory seed germination was recorded in the Chirpan-539 cultivar – 80.0%. The highest values were measured in cultivars Philipopolis and Natalia – 94.0% and 93.3% respectively. The herbicide had the weakest effect on the seed germination in these cultivars.

This shows that herbicides affected differently both the yield of cotton and the laboratory germination of cotton seeds. This should be taken into account when these seeds are used as seeds for sowing. Herbicides that reduce the seed germination should not be used in the respective cultivars which are grown for seed production.

The variance analysis of cotton seed germination (Table 3) revealed that herbicides had the greatest impact on this indicator - 20.0 % of the total variation. The reason for this is the phytotoxic action of some herbicides on cotton plants during vegetation. Years also had a great impact (12.6%), which was due to the different weather conditions throughout individual years of study. The degree of effect of cultivars is 12.4%.

The effect of years, cultivars and herbicides is very significant at p≤0.1. There is a significant interaction of herbicides with the conditions of years (AxB) - 4.0%, the effect of years with cultivars (AxC) is 6.8 % and that of herbicides with cultivars (BxC) – 29.5%. They are very significant at p≤0.1. There is also interaction between the three factors in the experiment (AxBxC) – 13.0%, also significant at p≤0.1.

Table 3. Analyses of variance for cotton seed germination

Source of variation	Degrees of freedom	Sum of squares	Influence of factor (%)	Mean squares
Total	215	13251.4	100	-
Influence of land	1	80.6	0.6	80.6***
Variants	107	13020.4	98.3	121.7***
Factor A - Years	2	1674.5	12.6	837.3***
Factor B - Herbicides	2	2654.1	20.0	1327.0***
Factor C - Cultivars	11	1640.5	12.4	149.1***
AxB	4	525.3	4.0	131.3***
AxC	22	898.4	6.8	40.8***
BxC	22	3908.5	29.5	177.7***
AxBxC	44	1719.1	13.0	39.1***
Pooled error	107	150.4	1.1	1.4

*p≤5% ; **p≤1%; ***p≤0.1%.

The analysis of variance for cotton seed germination showed that the herbicidal action strongly depends on weather conditions during the vegetation period. These are mainly temperature and rainfall after the herbicide treatment. Cotton cultivars also reacted differently when treated with herbicides Basagran 480 SL, Pulsar 40 and Express 50 SX in different years.

Based on the significant interactions of herbicide x year and cultivar x year the stability of manifestation of each variant was assessed with regard to laboratory germination of cotton seeds (Table 4). Shukla's stability variants σ_i^2 и S_i^2, Wricke's ecovalence W_i and the Kang's YS_i stability criterion were also calculated.

Shukla's stability variances (σ_i^2 and S_i^2) which take into account both linear and non-linear interactions uniquely assess the stability of variants. The variants with lower values are considered to be more stable because they interact less with environmental conditions. The negative values of the indicators σ_i^2 and S_i^2 are assumed to be 0. At reliably high values of either parameter - σ_i^2 or S_i^2 the variants are considered to be unstable. With Wricke's ecovalence W_i the higher the values of the indicator the more unstable the relevant variant. By using these three stability indicators we found that in vegetative treatment with the herbicide Basagran 480 SL the following cultivars: Trakia, Vicky, IPK-Veno, Avangard and Nelina were stable.

In treatment with Pulsar 40 the cultivars Helius, Trakia and Dorina were stable, while with the Express 50 SX the cultivars Avangard and Natalia were stable. The other variants had high instability, having Shukla's stability variance values σ_i^2 and S_i^2 and Wricke's ecovalence W_i high and significant. Instability was mainly due to the significant differences in the laboratory germination of cotton seeds in these variants throughout the years of the experiment, since herbicides had the strongest effect on them. In some of them there was instability of linear and non-linear type - significant values of σ_i^2 and S_i^2. In another part there was only instability of a linear type - significant value of σ_i^2, but the σ_i^2 и S_i^2 values not significant.

Table 4. Stability parameters for the variants for cotton seed germination with relation to years

Herbicides	Cultivars	$\bar{x}$	σ_i^2	S_i^2	W_i	YS_i
Basagran	Chirpan-539	64.2	28.1**	7.0*	55.4	-3
	Helius	97.3	18.2**	0.1	36.7	28+
	Trakia	89.8	3.0	1.1	7.9	16+
	Vicky	91.5	4.3	0.4	10.4	26+
	Philipopolis	96.7	68.6**	0.03	131.8	27+
	IPK-Veno	94.2	3.0	-0.09	8.1	32+
	Boyana.	97.3	18.2**	0.1	36.7	28+
	Avangard	90.7	-0.8	0.7	0.9	21+
	Natalia	98.2	15.8**	9.9**	32.3	31+
	Darmy	97.3	18.2**	0.1	36.7	28+
	Dorina	95.0	16.0**	-0.2	32.7	26+
	Nelina	94.8	2.9	1.1	7.9	33+
Pulsar	Chirpan-539	89.0	15.5**	1.6	31.7	1
	Helius	90.7	-0.8	0.7	0.9	21+
	Trakia	89.8	3.0	1.1	7.9	16+
	Vicky	89.2	28.1	7.0*	55.4	2
	Philipopolis	71.7	156.4**	0.7	297.7	-6
	IPK-Veno	90.7	154.0**	4.6	293.4	13+
	Boyana.	90.7	49.4**	23.4**	95.6	13+
	Avangard	81.5	25.0**	4.4	49.6	-7
	Natalia	89.8	78.2**	56.4**	150.0	8
	Darmy	89.2	28.1**	6.9*	55.4	2
	Dorina	74.8	3.0	1.1	7.9	-2
	Nelina	78.3	159.0**	2.3	302.7	-9
Express	Chirpan-539	80.0	16.1**	-0.1	32.7	-8
	Helius	89.2	110.3**	2.0	210.6	2
	Trakia	90.0	16.1**	-0.1	32.7	12
	Vicky	89.2	110.3**	2.0	210.6	2
	Philipopolis	94.0	15.5**	1.6	31.7	23+
	IPK-Veno	91.5	106.2**	3.7	202.9	18+
	Boyana.	85.7	49.4**	23.4**	95.6	-2
	Avangard	89.8	2.9	1.1	7.9	16+
	Natalia	93.3	-1.1	0.03	0.2	30+
	Darmy	83.3	159.0**	2.7	302.7	-6
	Dorina	91.7	16.9**	0.6	34.3	20+
	Nelina	84.0	15.5**	1.6	31.7	-4

In order to make an overall assessment of the effectiveness of each herbicide both its effect on seed germination and its stability - reaction of cotton varieties to it throughout the years – should be taken into account. Very valuable information about the technological value of variants is given by Kang's YS_i indicator for simultaneous evaluation of seed germination and stability based on the reliability of differences in the cotton seed germination and the variance of interaction with the environment. The value of this criterion is that by using non-parametric methods and statistical proof of differences, we obtain a generalized assessment ranking variants in descending order according to their economic value.

Kang's stability criterion YS_i taking into account both the stability and the value of seed germination gave negative values in cultivar Chirpan-539, treated with Basagran 480 SL, cultivars Philipopolis, Avangard, Dorina and Nelina, treated with Pulsar 40 and cultivars Chirpan-539, Boyana, Darmy and Nelina treated with Express 50 SX. They were characterized as the most unstable or the most sensitive to the herbicide regarding cotton seed germination. According to this criterion the most valuable in terms of cotton seed germination are cultivars Helius, Trakia, Vicky, Philipopolis, IPK-Veno, Boyana, Avangard, Natalia, Darmy, Dorina and Nelina with vegetative treatment with the herbicide Basagran 480 SL. In treatment with the herbicide Pulsar 40 the most valuable are cultivars Helius, Trakia, IPK-Veno and Boyana. In treatment with the herbicide Express 50 SX the most valuable are cultivars Philipopolis, IPK-Veno, Avangard, Natalia and Dorina. They combine high values and high stability with regard to laboratory seed germination throughout the years.

CONCLUSIONS

For the first time in the world, cotton cultivars resistant to herbicides Basagran 480 SL, Pulsar 40 and Express 50 SX were identified.

Herbicide Bazagran 480 SL had the highest phytotoxicity on seed germination in cultivars Chirpan-539.

Herbicide Pulsar 40 had the highest phytotoxicity on seed germination in cultivar Filipopolis.

Herbicide Express 50 SX had the highest phytotoxicity on seed germination in cultivar Chirpan-539.

From the viewpoint of technology for cotton growing, in terms of its impact on germination of cotton seeds, technologically the most valuable by foliar treatment with herbicide Bazagran 480 SL are cultivars Helius, Trakia, Viki, Filipopolis, IPK-Veno, Boyana, Avangard, Natalia, Darmy Dorina and Nelina.

For the first time in the world we established that in the vegetative treatment with herbicides, technologically the most valuable by foliar treatment with herbicide Pulsar 40 are cultivars Helius, Trakia, IPK-Veno, Boyana.

Technologically the most valuable by foliar treatment with herbicide Express 50 SX are cultivars Filipopolis, IPK-Veno, Avangard, Natalia and Dorina.

These variants combine high levels of the seed germination and high stability of this index during the years.

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