Determination of the tolerance of maize cross-breeds and their parent material to bromoxynil – an inhibitor of photosystem II

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Abstract

Herbicides can exert a phytotoxic effect on maize (*Zea mays* L.) plants. High maize cross-breeds tolerance to herbicides can be obtained by proper selection of parent material. Based on the knowledge of phenotypic signs of damage, it is possible to discard the parents that are the most susceptible to herbicides.The aim of the study was to compare the response of maize cross-breeds Dumka and Rywal, their parental forms and lines to bromoxynil – the herbicide belonging to PSII photosynthesis inhibitor group. Herbicide was used at the recommended (400 g ha^{-1}) and double (800 g ha^{-1}) rates. Bromoxynil phytotoxicity was reflected in changes in plant morphological traits, growth inhibition and green matter reduction of female parent of cross-breed Dumka and its component S64423-2. This indication was confirmed in the study of photosynthesis parameters –chlorophyll content and chlorophyll fluorescence in leaves. The cross-breed Rywal and their male parent proved to be tolerant to the bromoxynil.

Introduction

Abiotic stress disturbances in plant physiological processes are induced by external harmful factors such as spring frost or water deficit in soil. Numerous scientific papers point out that long-term use of herbicides with the same mode of action can be an important factor in decrease crop tolerance to herbicides (Duke, 1985; Forlani *et al*., 1995). Designation of particular herbicide as selective for a specific crop is relative, because even slight over-dosage, too high temperature or too high humidity and too early or too late date of spraying can be a reason of severe crop injury (Johnson and Young 2002; Olson *et al*., 2000). Precise determination of crop tolerance based on field experiments in variable habitats is important with regard to genetically diversified cultivars.

Maize susceptibility to herbicides can result in abnormal phenotypic changes *e.g.* delay in maturation, morphological or disease damages (Gołębiowska and Rola, 2003). In extremeweather conditionssuch as ground frost or drought, chronical injuries or diseases leading to deterioration in yield size and grain quality can be observed. The risk of herbicide derived damages can be reduced by the proper selection of parent material, the rejection of parents with high susceptibility to herbicides, hybrydization of parents with advantageous traits and also by the evaluation of breeding value based on progeny, i.e. cross-breed F1 (Bonis *et al*., 2004; Gołębiowska and Kowalczyk, 2007). This type of study was conducted with both flint and dent corn of maize (Nielsen *et al*., 2002; Choe *et al*., 2014).

Photosynthetic pigments in leaves are organized in photosystem which contains 200-300 assimilation particles that make them possible to absorb greater amount of energy. This energy is transmitted with chlorophyll a to the centre of photosystem and causes electron dislocation. Herbicides belonging to the group of photosystem II (PSII) inhibitors block the electrons flow and consequentlyplant withers (Smith, 1980; van Rensen, 1982; Sanders and Pallet, 1986; Bolharnordenkampe *et al*., 1989; Nielsen *et al*., 2007). Phytotoxicity of the foliar-applied herbicides belonging to the group of PSII inhibitors can be revealed as leaves discoloration and/or even permanent morphological changes such as total leaves withering. Additionally, it is strongly correlated to the weather conditions during the time of spraying (Tollenaar and Mihajlovic, 1991a).

One of the methods useful in the evaluation of cross-breeds and their parent material tolerance to bromoxynil can be fluorescence analysis based on the emission spectroscopy (Tollenaar *et al*., 1991b). During the processes of light absorption and transformation into chemical energy, part of the absorbed light which is not used for photochemical reactions, is reemitted as fluorescence. The effect of spectrofluorimetric analysis is the curve which describes the variation in fluorescence during the time of measurementdepending on genotype tolerance to this herbicide. The course of fluorescence curve can be modify when leaves are treated with a substance blocking electrons flow, *e.g.* the herbicide with mode of action involving the inhibition of PSII. The emission spectrum of the fluorescence and its variation during the measurement can be recorded using non-invasive (Kucharski and Rola, 2006).

The objective of this study was the determination of the tolerance of two maize cross-breeds, their parental forms and lines to bromoxynil (herbicide belonging to PSII inhibitors group) using the method based on visual symptoms of herbicide phytotoxicity, the determination of chlorophyll content and the variation in fluorescence curves.

Material and methods

Experimental plan

The pot experiment was carried out in glasshouse and each single series was repeated three times. The individual experiment was established for each of the tested cross-breeds of maize and included two factors: 1. genotypes (cross-breed and their parent material), 2. two doses of herbicide bromoxynil. It was arranged in the complete randomisation pattern with three replications.

Plant material

The first factor was maize cross-breeds (Dumka orRywal), their parent material (parental forms and lines).Three-line cross-breed Dumka contains female parent consisting of two lines (S64423-2 x S64411) and male parent S61328. It is medium-early cross-breed (FAO 230) with very good early vigour. The second one (Rywal) is also three-line cross-breed that contains female parent consisting of lines (S74449 x S74002) and male parent S61328. It is early cross-breed (FAO 210) with good early vigour.Maize seeds were sown into pots a diameter of 25 cm. Pots were filled with the mixture of peat and sand (2:1 v/v). The experiment were carried out under the following terms: photoperiod – 14/10h day/night, average day temperature 21°C±2°C, average night temperature 12°C±2°C, light intensity 400 $\mu mol/s^{-1}$. When the plants reached the stage of 1-st pair of true leaves, manual thinning was made and four plants per pot were left.

Table 1 - The scale used for the assessment of herbicide phytotoxicity

Degree	Size of plant injuries (%)
1	0
2	1-29
3	30-54
4	55-69
5	70-79
6	80-89
7	90-95
8	96-99
9	100

Herbicide treatment

The second factor was herbicide treatment including bromoxynil (commercial product Emblem Pro 385 EC) applied at two doses: the maximum recommended (400 g ha^{-1}), the double maximum (800 g ha^{-1}) and untreated (control).

Herbicide was applied at the growth stage of 4-6 leaves of maize. For each genotype, twelve plants were sprayed by bromoxynil at 400 g ha^{-1} and the same number were treated with bromoxynil at 800 g ha^{-1} (four plants per pot in trhee replication). The herbicide treatment was performed using a laboratory sprayer "Aporo" equipped with a beam with flat fan nozzle (XR 11003-VS). The nozzle was operated at the pressure of 0.25 MPa and speed 2.5 $km.h^{-1}$ to obtain a spray volume of 250 $l*ha^{-1}$.

Plant injury, plant height, fresh weight and chlorophyll content measurement

The evaluation of herbicide phytotoxicity was carried out one week after treatments. It was performed by the comparison of plant status on sprayed plots with untreated control using 9-degrees scale when 1 means no reaction and 9 means total crop damage. The scale is presented in Table 1.

Chlorophyll content in leaves was determined using Chlorophyll Content Meter CCM-200. This analysis was performed three weeks after herbicide treatments, on the middle part of the third leaf of each plant (four plants per pot=twelve plants per object were tested). Object means genotype treated with herbicide at particular dose. Just after the completion of this analysis, plant height was measured and then whole aboveground parts of plant were harvested and fresh weight was recorded.

Chlorophyll fluorescence analysis

Chlorophyll fluorescence was measured using fluorescence spectrophotometer F-2500 (HITACHI). Plant

Table 2 - Influence of bromoxynil on the cross-breed Dumka, its parental forms and lines

Genotypes (cross-breed, parental forms, lines)	Herbicides											
	Untreated (Control)				bromoxynil 400 g ha^{-1}				bromoxynil 800 g ha^{-1}			
	F 1:9	FW (g)	CCI	H (cm)	F 1:9	FW (g)	CCI	H (cm)	F 1:9	FW (g)	CCI	H (cm)
Dumka	1	33.6	29.9	35.6	3	32.1	22.3	26.2	4	30.4	22.9	24.2
Female parent S64423-2xS64411	1	25.6	29.3	22.3	4	19.3	7.3	10.2	5	15.4	12.9	9.6
Male parentS61328	1	31.7	28.9	24.8	4	31.0	16.3	14.8	4	29.3	9.6	14.2
Female lineS64423-2	1	23.6	19.1	21.6	6	12.1	8.3	8.2	6	11.4	8.9	6.2
Female lineS64411	1	30.7	19.9	25.8	3	30.2	15.3	14.4	3	29.7	10.6	13.2
LSD (0.05) for genotype x herbicide: FW = 2.69, H = 10.98												

F – phytotoxicity - susceptibility of plants to herbicide in scale 1:9 (the scale is explained in table 1), FW – fresh weight, CCI – chlorophyll content index, H – plant height

material were cross-breeds (Dumka, Rywal), their parental forms and lines taken from untreated plants grown under mentioned above conditions. For the analysis, the middle parts of twelve (four plants per pot in three replication) young and fully expanded leaves from each genotype were taken. Preparation of herbicide solutions and fluorescence measurement were performed according to the method described by Ducruet and Gasquez (1978) and modified by Kucharski and Rola, 2006). Just prior to fluorescence measurement, pieces of three leaves from each plant were soaked in aqueous solution of herbicide bromoxynil at the concentration of 100 μM while the remaining ones were soaked in distilled water (control). The samples of soaked leaves were placed in climate chamber, at the temperature of 20°C and light intensity of 350±10 μmol·m2·s^{-1}. After 3 hours, the light in climate chamber was switch off and the samples were further stored in the darknessfor one hour. Each sample was placed into the measuring chamber and chlorophyll fluorescence was recorded. As it is shown in Figure 1, in control samples, the onset of illumination caused a rapid increase in chlorophyll fluorescence to a maximum level (M) followed by decay to a steady-state fluorescence level (L) (Kucharski and Rola, 2006). In leaf samples of susceptible plants treated with herbicide, chlorophyll fluorescence raised immediately to a maximum level higher than M and thereafter remained constantly at this maximum level. For tolerant plant, the shape of the curve should be similar to control (untreated) samples (Kucharski and Rola, 2006).

Statistical analysis

The obtained data were subjected to ANOVA. When the differences were significant, the means were separated by multiple range Tukey's test, at a probability level of 0.05. The statistical analysis was performed using Statgraphic Plus 2.1 programme.

Results

Phytotoxic effect of bromoxynil on maize cross-breeds and their parent material

The greatest plant injuries (chlorosis and spots) were noted for cross-breed Dumka and its female parent S64423-2xS64411 and female line S64423-2 and also male parent S61328 (Table 2). These further reflected in significant, growth inhibition and fresh weight reduction in case of these genotypes (despite male parent). The greatest biomass and plant height reduction for female parent S64423-2xS64411 and female line S64423-2 treated with bromoxynil at the 800 g ha^{-1} was observed. High susceptibility of female parent and line S64423-2 contributed to revealance of this feature in cross-breed Dumka, however, it demonstrated less negative response to bromoxynil than parent material. The cross-breed Rywal and its two lines (S74002, S74449) treated with both recommended and double dose of bromoxynil showed slight, shortly-persisting discoloration of leaves and temporary growth retardation that did not finally affect plant height and above ground biomass of maize (Table 3). Similar symptoms appeared on plants of the cross-breed F1.

Table 3 - Influence of bromoxynil on the cross-breed Dumka, its parental forms and lines

Genotypes (cross-breed, parental forms, lines)	Untreated (Control)				bromoxynil 400 g ha^{-1}				bromoxynil 800 g ha^{-1}			
	F 1:9	FW (g)	CCI	H (cm)	F 1:9	FW (g)	CCI	H (cm)	F 1:9	FW (g)	CCI	H (cm)
Rywal	1	34.6	29.4	25.6	2	32.1	28.3	26.2	2	30.4	25.9	24.2
Female parent 74449xS74002	1	25.6	29.2	22.3	2	29.3	27.3	20.2	2	25.4	26.9	29.6
Male parentS61328	1	33.6	29.9	24.8	1	34.0	29.3	24.8	2	29.3	29.6	26.2
Female lineS74449	1	24.6	29.9	18.6	2	22.1	25.3	18.2	3	25.0	25.9	26.4
Female lineS74002	1	30.5	29.9	20.8	2	30.2	27.3	18.4	2	26.7	26.6	23.2
LSD (0.05) for genotype x herbicide: FW = 2.16, H = 3.84												

F – phytotoxicity - susceptibility of plants to herbicide in scale 1:9 (the scale is explained in table 1), FW – fresh weight, CCI – chlorophyll content index, H – plant height

Influence of bromoxynil on chlorophyll content index (CCI)

Changes in chlorophyll content resulted from the disruptions in photosynthesis process and they were correlated with the visual symptoms of bromoxynil effect. Based on CCI value, the highest chlorophyll content was observed for the untreated plants, that featured healthy and green leaves. For the cross-breed Dumka treated with both recommended and double dose of bromoxynil CCI values (22.3 and 22.9, respectively) were significantly lower as compared to untreated plants (29.9) (Table 2). In this case, low CCI values resulted from negative reaction to bromoxynil and from

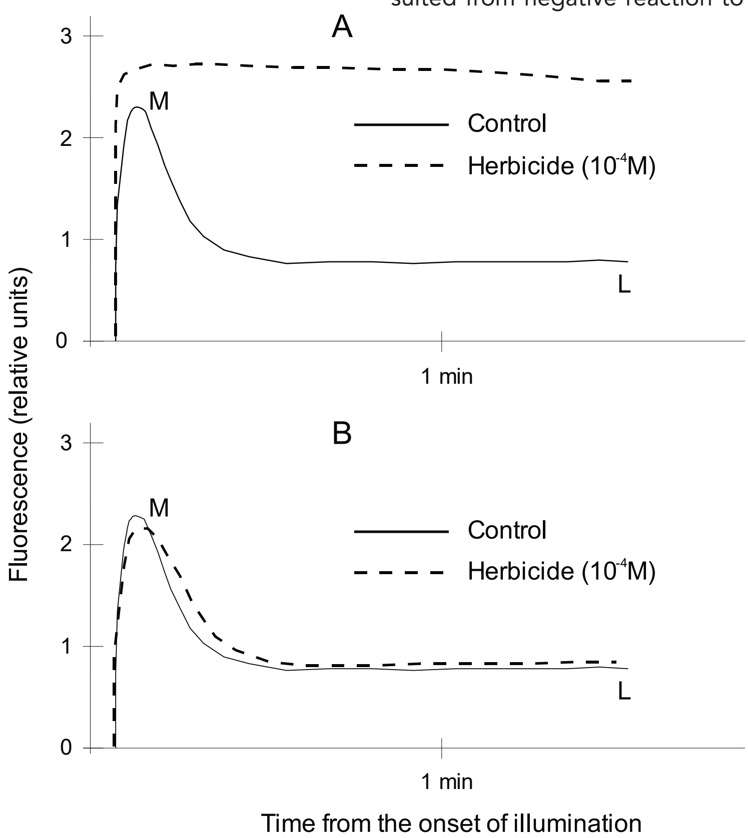

Fig. 1 - Descriptive model of fluorescence curve for susceptible (A) and tolerant (B) to herbicide maize cross-breeds (Kucharski and Rola, 2006) M – maximum level of fluorescence, L- steady-state of fluorescence

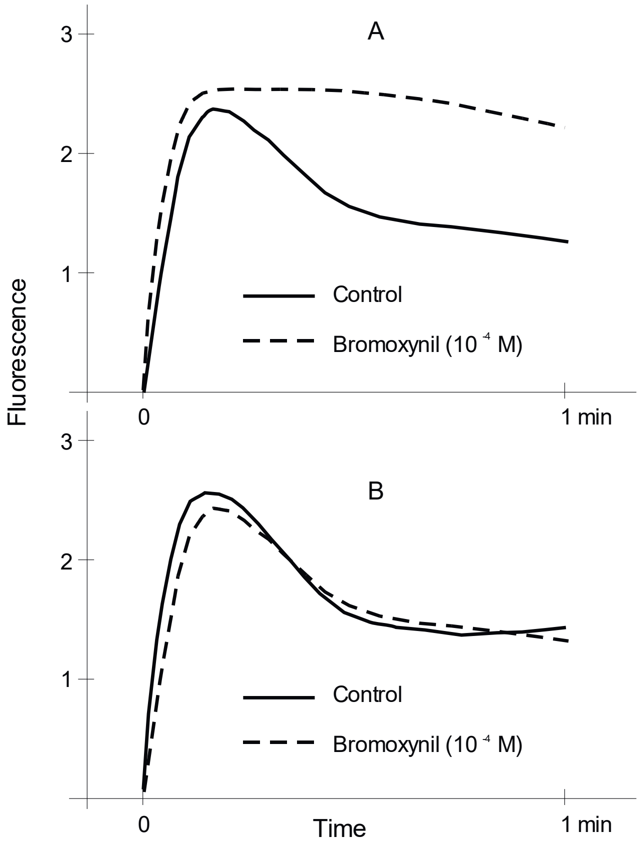

Fig. 2 -Fluorescence curve for susceptible cross-breed Dumka (A) and tolerant cross-breed Rywal (B). To be added more details about Fluorescence (relative units)

the susceptibility of line S64423-2 of female parent to this herbicide.

For the cross-breed Rywal treated with bromoxynil at both examined doses CCI was lower than for untreated maize and amounted to 28.3 for the recommended and 25.9 for double dose, although the differences were non-significant statistically (Table 3).

Influence of bromoxynil on chlorophyll fluorescence

Fluorescence in untreated plants reaches maximum and, afterwards, its value decreases very fast to stabilize after 30 seconds at the steady-state level (see: Material and Methods). Similar fluorescence course is expected for the plants tolerant to bromoxynil.On the other side, for plants susceptible to bromoxynil, fluorescence does not decrease during the measurement after reaching the maximum value. In this study, chlorophyll fluorescence for susceptible plantsof cross-breed Dumka, its female parent (S64423-2xS64411) and female line S64423-2 was maintained at maximum during the measurement or slightly decreased (Figure 2 A, 3 A, B). In turn, the shape of fluorescence curve for tolerant cross-breeds Rywalwas similar to those obtained for untreated plants, which confirms the high tolerance of both parents to bromoxynil.In this case, fluorescence decreased rapidly and its stabilization was achieved after 30 seconds (Figure 2 B).

The established criterions for the spectrofluorimetric evaluation of plant response to herbicide make possible to determine whether the examined cross-breed, its parental forms or lines are susceptible or tolerant. The results of the fluorescence analysis do not allow to interpret clearly, therefore they have to be confirmed by the results of visual symptoms and chlorophyll content in leaves. The results derived from the determination of chlorophyll content index (CCI) and chlorophyll fluorescence in plants of the examined cross-breeds, their parental forms and lines were in accordance with the results obtained from the evaluation of visual symptoms of herbicide phytotoxicity.

Discussion

Climate conditions are the most important factors affecting both herbicide efficacy and selectivity. Long-lasting period of low temperatures, too high or too low soil moisture and air humidity, as well as sunlight deficit slow down herbicide degradation in plants. It re-

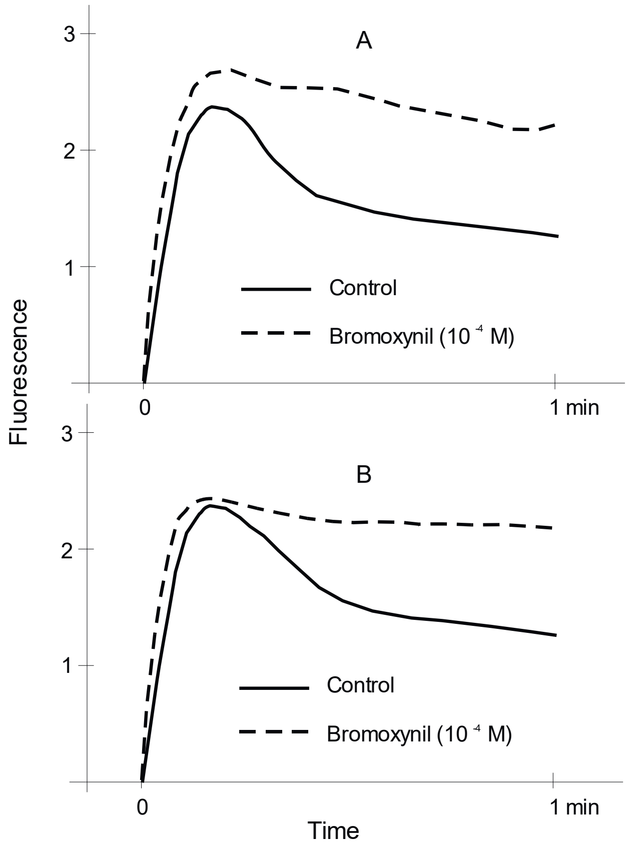

Fig. 3 -Fluorescence curve for susceptible female line S64423-2 (A) and female parent S64423-2 x S64411 (B) of cross-breed Dumka (Fig. 2 A)

sults in a greater herbicide phytotoxicity that reveals in the photosynthesis disruption in cultivated plants and weeds (Forlani *et al.*, 1995; Begna 2001; Xu *et al.*, 2007; Zablotowicz, 2009; Chen*et al.*, 2011). The consequence of these disturbances is change in chlorophyll content – the main photosynthetic pigment. When herbicide stress appears, the decrease of spectral reflectance in green range, chlorophyll content reduction andplants yellowing due to change in the proportion between chlorophylls and carotenoids are observed.Such a mechanism takes place in weeds when herbicide belonging to group "inhibitors of photosynthesis at PS II" is applied. However, the same reaction can be observed in sensitive maize cultivars (Bohme *et al.*, 1981).

Cross-breeds of maize are more tolerant to herbicides than their parent material, as reported by Gołębiowska and Rola (2010) that proved diversified response of some cross-breeds to nicosulfuron and rimsulfuron. The first study in this area was conducted by Green and Ulrich (1993); they evaluated the effect of three sulfonylurea herbicides, i.e. nicosulfuron, tifensulfuron-methyl and primisulfuron-methyl. Among thirty six examined lines, eleven were susceptible to herbicides, while only one from twenty eight of the examined cross-breeds showed high susceptibility. It responded to herbicides by significant growth inhibition, while the remaining cultivars exhibited only slight and temporary chlorosis (Green and Ulrich, 1993). Similar results were achieved in a study with lines of sweetcorn that were susceptible to the mixture of nicosulfuron with bromoxynil (Corbett *et al.*, 2005). Gołębiowska and Kowalczyk (2007) proved that the male parent of cross-breed Kokaresulted highly susceptible to rimsulfuron and has shown significant decrease in plant height, grain and cob yield and in the weight of 1000 grain. High susceptibility appeared also in F1 generations which werecross-breed Koka. For contrast, maternal form of the mentioned cross-breed responded to rimsulfuron by slight and temporary chlorosis. Similar question was investigated by Tollenaar and Mihajlovic (1991a) who reported that greater discoloration of maize leaves was provoked by atrazine used in the mixture with mesotrione (herbicide from group of pigments inhibitor) than by atrazine used alone. However, plant damages were temporary and did not affect the yield loss as compared to those provoked bymesotrione applied single (Tollenaarand Mihajlovic, 1991a).

A study on three genetically diversified maize cross-breeds and their response to bromoxynil was carried out by Bolharnordenkampe *et al.* (1989) in order to find out whether their grain yield was correlated with the tolerance to bromoxynil. The measurements of chlorophyll fluorescence allowed to calculate the index of photosynthesis efficiency and its relationship with the grain yield. High index of photosynthesis efficiency was correlated with high grain yield.

Numerous reports on the occurrence of low tolerance of maize cultivars to bromoxynil contributed to search for the strategies to discover new herbicides and/or tolerant crop to achieve the highest herbicide safety for crop. Bromoxynil is the inhibitor of photosystem II (PSII) and it is used for broad-leaved weeds control. There are currently available some synthetic counterparts of bromoxynil which have been evaluated with respect to the effect on weeds in soyabean or cotton. Although both bromoxynil and all its counterparts inhibit electrons flow in PSII, it is considered that the new discovered counterparts are more efficient in weed control and, at the same time, less phytotoxic to crop than bromoxynil (Corbett *et al.*, 2004).

Conclusions

Plant breeding process implements appropriate procedures to obtain cross-breeds with diserable traits, among others tolerance to biotic or abiotic stresses. However cross-breeds tolerance to herbicides is not feature targeted by maize breeding programmes. This research investigated this issue including two maize cross-breeds and their parent material as well as one herbicide belonging to the group of photosynthesis inhibitors. The results of this study allow to statethat the knowledge about tolerance of maize parent material to herbicides make possible to predict this feature for cross-breeds. In this study, cross-breed Dumka demonstrated susceptibility to bromoxynil and it was also noted for its female parent S64423-2xS64411 and line S64423-2, although cross-breed was less sensitive than parent material.

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