Annals of Warsaw University of Life Sciences – SGGW
Horticulture and Landscape Architecture No 38, 2017: 35–41
(Ann. Warsaw Univ. of Life Sci. – SGGW, Horticult. Landsc. Architect. 38, 2017)
DOI 10.22630/AHLA.2017.38.4

Suitability of selected Polish field bean cultivars *(Vicia faba* var. *minor*) for the root tip genotoxicity assay (*Vicia* RTA)

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Abstract: *Suitability of selected Polish field bean cultivars (*Vicia faba *var.* minor*) for the root tip genotoxicity assay (*Vicia *RTA).* Different cultivars of the same species may vary in sensitivity to toxic or genotoxic factors; therefore the cultivar of *Vicia faba* var. *minor* applied as a bioindicator in the root tip genotoxicity assay (RTA) may affect the results of the test. The aim of our study was to evaluate the parameters relevant to genotoxicity test performance and results in certain Polish cultivars of field beans, and to select the most appropriate bioindicator for the *Vicia* RTA. Six cultivars were used in the experiment: three low-tannin – 'Amulet', 'Olga' and 'Kasztelan' – and three high-tannin – 'Nadwiślański', 'Kodam' and 'Neptun'. We concluded that: the cultivar of the test plant in the *Vicia faba* RTA may significantly affect the test result, and therefore to obtain comparable data, the same cultivar should be used as the bioindicator; 'Kodam' and 'Amulet' seem to be the worst choices, because of the relatively high percentage of spontaneous chromosomal aberrations and lowest sensitivity to mitodepressive and genotoxic agents; the best recommendation appears to be the cultivar 'Olga', with the lowest spontaneous chromosomal aberration percentage, highest sensitivity to genotoxic agents and relatively high sensitivity to mitodepressive agents.

Key words: genotoxicity, *Vicia faba*, cultivar, root tip assay RTA

INTRODUCTION

Several methods using plants as bioindicators are recommended as efficient and relatively low-cost tools for genotoxicity assessment of environmental samples [Ma 1999, Grant 1999, Leme and Marin-Morales 2009]. *Allium cepa* and *Vicia faba* root tip assays (RTA) are quite often used for this purpose [Ma et al. 1995, Smaka-Kincl et al. 1996, Evseeva et al. 2003, Feretti et al. 2012]. The *Vicia* RTA seems to be a more convenient method, because seedling roots of field beans are easier to obtain than the adventitious roots of onion bulbs, and the storage of seeds is much less demanding. Therefore this method is preferred for genotoxicity assessment of solid [Minissi et al. 1998, Cotelle et al. 2015] and liquid samples [Zhong et al. 2001, Sang and Li 2004, Abdel-Migid et al. 2007]. Some authors use broad beans (*Vicia faba* var. *major*) [Cotelle et al. 2015, Correa et al. 2016] and some field beans (*Vicia faba* var. *minor*) [Obidoska 2007, Valencia-Quintana et al. 2016]. In 2013 the *Vicia faba* micronucleus test with broad beans (*V. faba* var. *major*) was standardised and described in the international protocol ISO 29200 "Assessment of genotoxic effects on higher plants – *Vicia faba* micronucleus test". The protocol gives instructions concerning performance of the test, but the cultivar of the test plant is not specified, even though it is known that the cultivars of some species, including *Vicia faba* [Samborska-Ciania

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1993], may vary in sensitivity to toxic or genotoxic factors such as lead [Lei et al. 2006] or cadmium [Rahoui and Chaoui 2008]. This may affect test results [Cecchi et al. 2007]. Therefore, the aim of our study was to evaluate the parameters relevant to genotoxicity test performance and results in six Polish cultivars of field beans (*Vicia faba* var. *minor*) and to select the most appropriate bioindicator for the *Vicia* RTA.

MATERIAL AND METHODS

Six Polish cultivars of field beans (*Vicia fab* var. *minor*) were used in the experiment: three low-tannin cultivars – 'Amulet', 'Olga' and 'Kasztelan' – and three high-tannin ones – 'Nadwiślański', 'Kodam' and 'Neptun'. The seeds, obtained from Polish plant breeding stations in Strzelce, Tulce and Choryń, were of the same age (2 years) and were stored in the same conditions. To assess their suitability for root tip assay, an experiment was conducted in two parts. In the first part four parameters were evaluated in control conditions: seed germination, the ability of seedlings to produce lateral roots (test organs), mitotic index (MI), and the level of spontaneous chromosomal aberrations in anaphases and telophases (AAT). In the second part the seedlings of the six cultivars were exposed to a genotoxic environmental sample to assess their sensitivity.

In the first part of the experiment *Vicia* seeds were sterilised for a few seconds in boiling water and placed in Petri dishes. For each cultivar, eight seeds were sown and the tests were replicated three times. Seeds were incubated between two layers of moist filter paper in darkness and at room temperature (20°C) for 5 days. After that time, the number of germinating seeds was calculated and the tips of main roots, longer than 2 cm, were cut to stimulate the emergence of lateral roots. After another 3 days the number of lateral roots longer than 2 cm was counted. The roots from each cultivar were cut off, fixed in Carnoy's solution (75% ethanol, 25% glacial acetic acid) and stored in 70% ethanol. Before slide preparation they were placed in distilled water for 10 min and subsequently stained with aceto-orceine with 1N HCl (9 : 1). The root tips were used for microscopic slide preparation.

In the second part of the experiment the seedlings with lateral roots, prepared as described above, were incubated for 24 h in beakers with tap water (control) and with water from the Vistula river, collected in central Warsaw at the storm water collector outlet. The genotoxicity of the environmental sample had been previously detected with the *Allium cepa* RTA. After incubation the lateral roots were cut off, and treated as described above.

All microscopic observations were carried out under an optical microscope (480× magnification). Mitotic index (MI) was expressed as the percentage of dividing cells per 1,000 scored cells (mitotic and interphase), and the percentage of anaphases and telophases with chromosomal aberrations (AAT), such as bridges, fragments and vagrant chromosomes, was calculated in 200 anaphase and telophase cells. Mitodepressive and genotoxic percent effects (PE) were calculated according to Moody and Miller [2005]. The presented data are averages of three replications.

Statistica 12 (StatSoft Polska Sp. z o.o.) was used for statistical analysis. The results were subjected to one-way analysis of variance (ANOVA) and subsequently to Tukey's test ($p \leq 0.05$) to distinguish homogeneous groups of means.

RESULTS AND DISCUSSION

In the first part of the experiment *V. faba* cultivars were tested in control conditions. Parameters characterising seed germination and the potential for lateral root production were similar for all six cultivars; the observed differences were not statistically significant (Table 1). The percentage of germinated seeds ranged from 76% ('Amulet') to 92% ('Kasztelan'). The potential for lateral root production was 9–13 roots per seedling. No differences in germination and seedling growth were observed low- and high--tannin cultivars, although some authors have reported that high-tannin cultivars germinate better and the seedlings exhibit higher vigour [Kolasińska and Wiewióra 2002]. In the control conditions of growth, there were also no differences between cultivars in mitotic indices (8.9–10.4%), but spontaneous chromosomal aberrations in anaphases and telophases varied significantly (Table 1), the highest levels (over 2%) being observed in 'Kodam' and 'Amulet' and the lowest (0.70%) in 'Olga'. Low spontaneous aberration frequency is an important property for a genotoxicity testing bioindicator [Nefic et al. 2013].

In the second part of the experiment the six cultivars were exposed to a mitodepressive and genotoxic river water sample. Two of them ('Kodam' and 'Amulet') did not indicate the mitodepressive effect, while the other four did (Fig. 1). The mitodepressive percent effect (PE) was similar in 'Nadwiślański', 'Kasztelan' and 'Olga', but a little lower in 'Neptun' (Table 2). All six cultivars detected the genotoxicity of the environmental sample (Fig. 2), but some differences in their sensitivity were observed: 'Kodam' and 'Amulet' were consistently

TABLE 1. Features important for a test plant in the *Vicia* RTA in six Polish cultivars of *Vicia faba* var. *minor*, measured in control conditions (tap water, darkness, room temperature)

Parameter	'Amulet' (L)	'Olga' (L)	'Kasztelan' (L)	'Nadwiślański' (H)	'Kodam' (H)	'Neptun' (H)
Germinated seeds (%)	76 a	87 a	92 a	82 a	84 a	85 a
Number of lateral roots per seedling	11 a	11 a	12 a	13 a	13 a	9 a
Mitotic index – MI (%)	10.4 a	9.3 a	10.0 a	9.7 a	8.9 a	10.3 a
Spontaneous chromosomal aberrations – AAT (%)	2.7 b	0.7 a	1.3 ab	1.5 ab	2.3 b	1.3 ab

Means with the same letter in a given row do not differ significantly.
L – low tannin; H – high tannin.

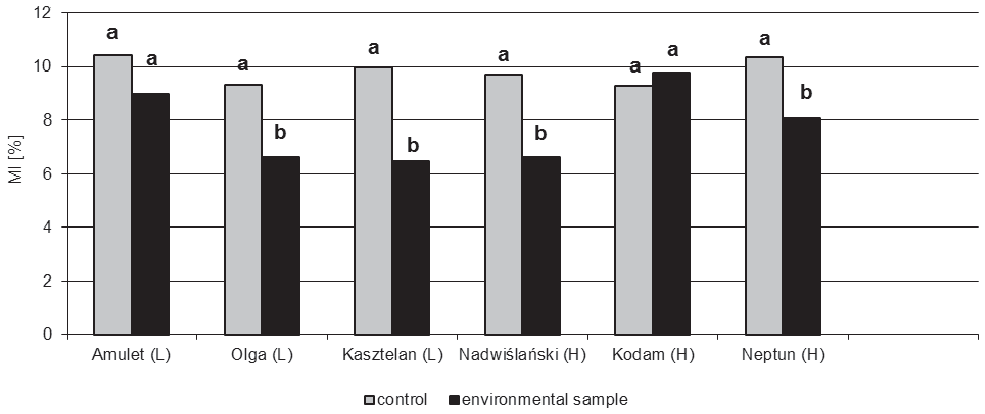

FIGURE 1. Influence of river water (environmental sample) on mitotic indices in root meristematic cells of six cultivars of *Vicia faba* var. *minor.* Means with the same letter for a given cultivar do not differ significantly

TABLE 2. Sensitivity of six Polish cultivars of *Vicia faba* var. *minor* to mitodepressive and genotoxic agents occurring in a river water sample, evaluated by the percent toxic effect (PE) for the mitotic index and for chromosomal aberrations in ana-telophase

Parameters/Cultivars	'Olga'	'Kasztelan'	'Nadwiślański'	'Neptun'	'Amulet'	'Kodam'
Mitodepressive percent effect (PE%)	28.7 ab	34.0 a	31.7 a	22.0 b	mnd	mnd
Genotoxic percent effect (PE%)	94.4 a	83.4 b	83.3 b	87.1 b	73.3 c	70.9 c

Means with the same letter in a given row do not differ significantly.
mnd – mitodepressive effect not detected.

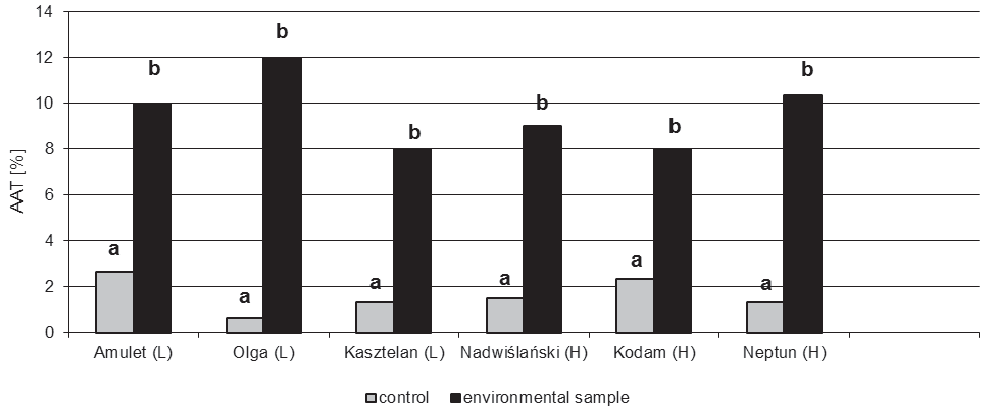

FIGURE 2. Influence of river water (environmental sample) on chromosomal aberrations in ana-telophases (AAT) in root meristematic cells of six cultivars of *Vicia faba* var. *minor*. Means with the same letter for a given cultivar do not differ significantly

the least sensitive cultivars, and 'Olga' the most reactive (Table 2). The differences in sensitivity to genotoxic agents between cultivars may affect the results of tests [Cecchi et al. 2006]. River waters, especially in large cities (such as Warsaw in this case), contain cadmium, chromium, copper, mercury, nickel, lead, zinc, and also organic genotoxic pollutants [Thornton et al. 2001], and consequently in the experiment the genotoxicity of the Vistula water sample was detected by even the least sensitive cultivars. However, to prevent non-detection of genotoxic environmental samples, a good bioindicator should react even to agents having lower genotoxicity or occurring in lower concentrations. In our experiment the most sensitive cultivar was 'Olga'. In our opinion this cultivar can be recommended for the root tip genotoxicity assay (RTA).

CONCLUSIONS

1. The cultivar of the test plant used in the *Vicia faba* root tip assay may significantly affect the test result; therefore, to obtain comparable data, the same sensitive cultivar should be used as the bioindicator.
2. 'Kodam' and 'Amulet' seem to be the worst choices among the six tested cultivars, because of their relatively high percentage of spontaneous chromosomal aberrations and lowest sensitivity to mitodepressive and genotoxic agents.
3. The best recommendation for a bioindicator for the *Vicia faba* root tip assay is the cultivar 'Olga', which has the lowest spontaneous chromosomal aberration percentage, the highest sensitivity to genotoxic agents and a relatively high sensitivity to mitodepressive agents occurring in the environmental sample of river water.

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Streszczenie: *Przydatność wybranych polskich odmian bobiku* Vicia faba *var.* minor *do testu genotoksyczności z zastosowaniem stożków wzrostu korzeni (*Vicia – *RTA).* Odmiany tego samego gatunku mogą wykazywać zróżnicowaną wrażliwość na czynniki toksyczne i genotoksyczne. Nie jest zatem wykluczone, że odmiana bobiku (*Vicia faba* var. *minor*), wykorzystana jako bioindykator w teście genotoksyczności z zastosowaniem stożków wzrostu korzeni (RTA), może wpływać na otrzymany wynik. Celem naszych badań była ocena parametrów charakteryzujących wybrane polskie odmiany bobiku, istotnych dla przebiegu i rezultatu testu, a także rekomendacja najbardziej odpowiedniej do zastosowania w tej metodzie. Oceniono sześć kultywarów: trzy niskotaninowe (‘Amulet’, ‘Olga’, ‘Kasztelan’) i trzy wysokotaninowe (‘Nadwiślański’, ‘Kodam’, ‘Neptun’). Stwierdzono, iż odmiana rośliny testowej może znacząco wpływać na wynik testu *Vicia* RTA, a zatem aby otrzymywać porównywalne rezultaty, należy używać tej samej odmiany; ‘Kodam’ i ‘Amulet’ wydają się być najmniej odpowiednie z powodu stosunkowo dużego odsetka spontanicznych aberracji chromosomowych i najmniejszej wrażliwości na czynniki mitodepresyjne i genotoksyczne; godna polecenia jest ‘Olga’ o najmniejszym odsetku spontanicznych aberracji chromosomowych, największej wrażliwości na czynniki genotoksyczne i stosunkowo dużej wrażliwości na czynniki mitodepresyjne.