Bulgarian Journal of Agricultural Science, 7 (2001), 255-260
National Centre for Agrarian Sciences

Gliadin Analysis for Identification of 1BL/1RS Wheat-Rye Translocations and for Assessment of Protein Patterns in Aluminium Tolerant Genotypes

S. D. STOYANOVA, D. B. BOYAJIEVA and Ch. Ch. PHYLIPOV
Institute for Plant Introduction and Genetic Resources, BG-4122 Sadovo, Bulgaria

Abstract

STOYANOVA, S. D., D. B. BOYADJIEVA and Ch. Ch. PHYLIPOV, 2001. Gliadin analysis for identification of 1BL/1RS wheat-rye translocations and for assessment of protein patterns in aluminium tolerant genotypes. *Bulg. J. Agric. Sci.*, 7: 255-260

Wheat germplasm of different origin (58 accessions) were evaluated by acid gliadin electrophoresis to describe genotypes possessing 1BL/1RS translocation. Wheat cv. Marquis was used to detect the relative mobility (RM) of gladin components. As a marked for 1RS group of bands in the area of w-gliadins was used wheat cv. Kavkaz. Aluminium tolerance was described both for 1BL/1RS translocated wheat and for non-translocated. The effect of aluminium was presented by root growth indexes at four Al-media (0 mM, 0.18 mM, 0.36 mM, 0.72 mM and 1.4 mM). Cluster analysis of gliadin components was carried out to specify the association between gliadin patterns and tolerance to toxic aluminium. 1BL/1RS translocated wheats were found to be more tolerant to Al-toxicity.

Key words: wheat, 1BL/1RS translocation, gliadin, electrophoresis, aluminium tolerance

Introduction

Aluminium toxicity is considered the most important metal toxicity problem on acid soils. As nearly half of the non irrigated arable lands in the world are acid soils, Al toxicity is a severe problem for crop production (Clark, 1982; Barchelo et al., 1996). One of the proposed strategies for crop performance under subsoil acidity is to stimulate the breeding of Al-tolerant genotypes (Shainberg et al., 1996).

Usually the firs observed symptoms of Al injury is an inhibition of root growth as a result of inhibited root elongation (Barchelo et al.,1996; Vazquez et al., 1999). Although much work has been done on the mechanism of Al-toxicity, the differences between species in their ability to resist of tolerance the Al-present in soil is still a point of discussion (Massot et al., 1999). Rye is known as more tolerant than wheat to aluminium toxicity because could be successfully growth in acid soil. High tolerance to aluminium toxicity is presented for triticale cv. Vihren which might due to the rye genes (Tzvetkov, 1994). It is reported that the developed in CIMMYT line 'Alondra S' bearing 1BL/1RS wheat-rye translocation along with the other characters possesses a moderate tolerance to aluminium (Rajaram et al., 1983).

Wheats with 1BL/1RS wheat-rye translocation chromosome have been used to transfer genes for enhanced agronomic performance from rye to wheat (Zeller, 1973; William et al., 1992). Associated with 1RS chromosome are several decease resistance traits (MacIntosh, 1983). In our previous is establisned that translocated wheats are more tolerant to toxic aluminium (Stoyanova et al., 2000). However 1BL/1RS wheat-rye translocated genotypes have been announced about for a long time and widespread little is known for their survival under unfavourable Al concentration.

This study is designet to evaluate the specific inhibition of root growth induced by toxic Al and its association with gliadin spectra of translocated or non-translocated wheats.

Materials and Methods

Germplasm. A total of 58 wheat accessions are evaluated. 10 are received by free-exchange abroad and 48 originated from the Bulgarian breeding programs (10 wheat cultivars and 38 new selected candidate cultivars and lines).

Electrophoresis. Gliadins are extracted from single seeds with 70% ethanol and fractionated by acid (pH 3.1) polyacrylamide gel electrophoresis (A-PAGE). Gliadin proteins are separated in a 1 mm thin vertical stab, at 5 mA per slot 7 h at 20 °C (Stoyanova, 1991). As a marked for the group of gliadin components 1B3 = 1BL/1RS is used wheat cv. Kavkaz. Wheat cv. Marquis is used to describe the relative mobility (RM) of gliadin components. Electrophoregrams are scaned directly by Epson GT7000 with Corel Photo Haus.

Root growth. Seeds of the evaluated cultivars are surface sterelised in 10% calcium hypochlorite and then set 24 h for pre-soaking in distillate water. The imbibed seeds are moved in petry-dishes over moistened filter-paper layers for germination. 48 h old seedlings with uniform primary root are set in 2 cm distance from the upper edge of the filter-paper rolls (paper size 15x20 cm). Ten seeds in four replicates per case are used for further measurement.

Screening for Al-toxicity is carried out in a media (pH = 4.0) with increasing Al concentrations added to nutrient solution of the following composition (in mM): 4.0 $CaCl_2$, 6.5 KNO_3, 2.5 $MgCl_2$, $6H_2O$, 0.12 $(NH_4)SO_4$, 0.4 NH_4NO_3. The nominal Al concentration in five rates is achieved by adding $Al_2(SO)_3$ in the nutrient solution as follow: 0 mM, 0.18 mM, 0.36 mM, 0.72 mM and 1.4 mM.

The wrapped filter rolls with 48 h seedings are transferred in glasses and dipped in 5 cm of the solution. Root growth is measured 5 days later, The negative effect of aluminium is presented as an index of root growth in different media compared to the growth of non-treated seeds (0 mM).

Statistics

The differences between treatments are determined by T-test at 5% level of significance.

Gluster analysis (ward's method, Euclidean distances) is done to describe the grouping of wheat genotypes and associations between the gliadin components for which the comulative frequency is higher than 3.

Result and Discussion

Aluminium tolerance in relation to presence of 1BL/1RS translocation

The patterns of alcohol-soluble seed protein of the 1BL/1RS translocation are different from those of wheat cultivars/lines without any 1RS-translocation (Figure 1). According to Lawrence and Shepherd (1981) the typical 1RS group of bands which appears in the area of ω-gliadins is a product of Sec-1 locus. This group of bands is observed in 19 wheat accessions (Figure 1, Table 1). The A-PAGE allows to distinguish 39 not translocated wheat cultivars or lines. The screening by gliadin patterns is the basis

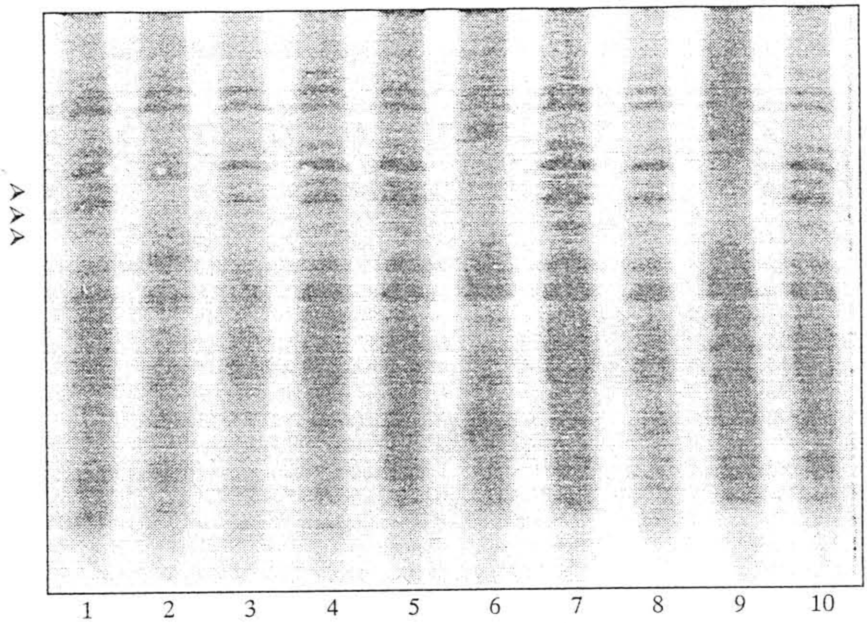

Fig. 1. Gliadin electrophoregrams observed in wheat cultivars by vertical A-PAGE for identification 1BL/1RS translocation (direct scan of the gel). The arrows present the group of ω-gliadins (RM25, RM29 and RM33) in cv. Kavkaz (line 1) used as a marker for 1BL/1RS translocation:
a) cultivars/lines bearing 1BL/1RS translocations: (3) Alondra "S"; (4) Shabla; (5) D380; (7) K88/Orfei; (8); Yana (10);
b) cultivars/lines lacking translocation: (2) Opata 58; (6) Momchil; (9) Sadovo 552;

to separate two genotype groups contrasting by presence of 1BL/1RS translocation.

Further analyses of root growth indexes at a media with increasing concentration of Al show large variation between genotypes. The statistical computation of the data present the mean values and standard deviation between two compared groups (Table 1). The mean values of root growth indexes in four Al-media are higher for genotypes bearing 1BL/1RS translocation however significant differences are confirmed statistically at two media only (0.36 mM and 0.72 mM).

At the lowest Al-concentration (0.18 mM) the phytotoxic effect is slight. As a result in that cases root growth is similar both for translocated and for non-translocated wheats. For seedlings treated in a media with 1.4 mM Al there is a tendency for narrowing between the two compared statistical rows. This effect probably due to approaching the upper limit in the evaluated scale of Al-concentration. Further increase of Al would affect stronger the seedling so the differences in phytotoxic reaction should be vague.

The results discussed here illustrate that the presence of 1BL/1RS translocatios relates to the tolerance of wheat genotypes to toxic aluminium.

Associations between gliadin spectra and aluminium tolerance of wheat

The cluster analysis of gliadin patterns results in some association groups (Figure 2). Cluster 1 includes 7 variables and tree of them (RM25, RM29 and RM33) have lower distances from the respective cluster centre (Table 2a). These three components clustered

Table 1
Statistical evaluation of differences in root growth indexes in Al-media for weath germplasm contrasting by presence/absence of 1BL/1RS translocations

Cultivars/Lines	Al concentration, mM				Cultivars/Lines	Al concentration, mM			
with 1BL/1RS	0.18	0.36	0.72	1.4	lacking 1BL/1RS	0.18	0.36	0.72	1.4
1. Alondra S	0.57	0.59	0.58	0.49	20. Tepoca	0.73	0.64	0.43	0.30
2. Bacanora 88	0.55	0.46	0.48	0.45	21. Rayon	0.79	0.67	0.39	0.31
3. Mochis 88	0.86	0.88	0.79	0.51	22. Opata 85	0.65	0.56	0.38	0.30
4. Seri 82	0.69	0.53	0.41	0.29	23. Tarasque	0.65	0.41	0.38	0.27
5. Burgus 2/Rdisn 5481	0.80	0.77	0.67	0.39	24. Super flatua	0.65	0.41	0.45	0.43
6. Shabla)1	0.71	0.61	0.49	0.33	25. Prelom)1	0.60	0.57	0.44	0.30
7. KC 549)2	0.82	0.75	0.69	0.49	26. Sadovo 552)1	0.70	0.57	0.41	0.38
8. K88/Orfei)2	0.89	0.82	0.73	0.52	27. Momchil)1	0.86	0.60	0.34	0.28
9. D380)2	0.75	0.64	0.58	0.50	28. K864/Yantar)2	0.58	0.47	0.44	0.36
10. D378R)2	0.92	0.85	0.74	0.65	29. Priaspa)1	0.55	0.51	0.35	0.28
11. D808)2	0.47	0.42	0.55	0.36	30. Yantar)1	0.90	0.94	0.90	0.90
12. D388 R)2	0.85	0.75	0.79	0.53	31. KC545)2	0.75	0.52	0.41	0.35
13. Boriana)1	0.87	0.77	0.80	0.52	32. KC60)2	0.97	0.69	0.72	0.40
14. Yana)1	0.77	0.79	0.61	0.60	33. KC436)2	0.57	0.41	0.33	0.37
15. KC768/4006)2	0.55	0.56	0.50	0.29	34. Predela)1	0.79	0.77	0.63	0.55
16. KC745/4204)2	0.53	0.49	0.53	0.21	35. Gracia)1	0.79	0.55	0.50	0.38
17. D511/4405)2	0.56	0.55	0.32	0.26	36. Record)1	0.89	0.52	0.46	0.45
18. D873/4489)2	0.65	0.76	0.51	0.39	37. KC290)2	0.81	0.71	0.73	0.64
19. LS317/4433)2	0.88	0.86	0.77	0.73	38. H21)2	0.74	0.50	0.36	0.27
					39. LS61)2	0.70	0.59	0.66	0.51
					40. D272)2	0.90	0.70	0.65	0.50
					41. D525)2	0.56	0.60	0.39	0.43
					42. D507)2	0.51	0.44	0.33	0.21
					43. D163)2	0.62	0.50	0.45	0.28
					44. KC746)2	0.78	0.78	0.62	0.48
					45. 18-32)2	0.66	0.41	0.27	0.27
					46. KC/Zornitza)2	0.66	0.48	0.40	0.27
					47. KC746/4204)2	0.77	0.59	0.59	0.42
					48. KC 747/4204)2	0.62	0.69	0.52	0.39
					49. KC749/4204)2	0.46	0.40	0.30	0.34
					50. D288/4345)2	0.85	0.78	0.75	0.43
					51. D913/4494)2	0.60	0.45	0.42	0.31
					52. LS285/4400)2	0.58	0.41	0.33	0.34
					53. LS5/4498)2	0.85	0.58	0.43	0.32
					54. LS316/4433)2	0.66	0.57	0.35	0.30
					55. LS318/4433)2	0.73	0.70	0.69	0.62
					56. LS284/4400 R)2	0.73	0.59	0.65	0.35
					57. K609/3380 R)2	0.61	0.59	0.48	0.41
					58. C50)2	0.83	0.73	0.71	0.43
Mean root-growth index ± stand. dev.	0.72 ± 0.15	0.67 ± 0.15*	0.60 ± 0.14*	0.44 ± 0.13		0.71 ± 0.12	0.58 ± 0.13*	0.49 ± 0.15*	0.39± 0.13
T-value	0.19	2.21*	2.72*	1.22					
p	0.844	0.030*	0.085*	0.220					
F-ratio varians	1.54	1.30*	1.10*	1.07					
p – varians	0.25	0.48*	0.85*	0.82					

)1 Bulgarian wheat cultivars;)2 new lines candidate cultivars created in IPGR – Sadovo; non-assigned lines and cultivars are received by free exchange abroad; * differences between two rows (1B/1R and 1B) are statistically significant at $P < 0.05$

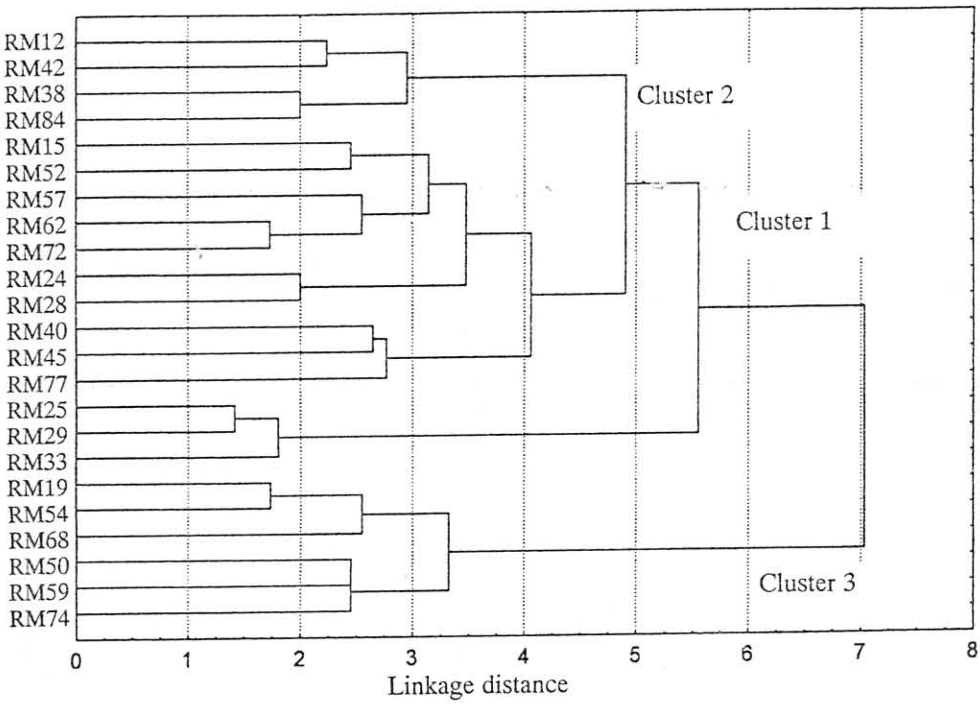

Fig. 2. Dendogram for classification of wheat accessions in relation to gliadin patterns (Ward's method, Euclidean distances)

Table 2
Cluster analysis and distances from respective cluster centre for gliadin components more frequently observed in evaluated wheat germplasm

a) Cluster 1 of 7 variables

Distances from respective cluster centre

Component	RM25	RM29	RM33	RM40	RM45	RM52	RM77
Distance	0.35	0.32	0.39	0.49	0.48	0.43	0.41

b) Cluster 2 of 9 variables

Distances from respective cluster centre

Component	RM12	RM15	RM24	RM38	RM42	RM57	RM62	RM72	RM84
Distance	0.40	0.44	0.37	0.48	0.45	0.43	0.39	0.42	0.36

c) Cluster 3 of 7 variables

Distances from respective cluster centre

Component	RM19	RM28	RM50	RM54	RM59	RM68	RM74
Distance	0.38	0.43	0.40	0.34	0.40	0.40	0.40

together illustrates the typical 1BL/1RS group of bands (Figure 1). Gluster 2 is compound of 9 variables (Table 2b). Low distances are described for variables RM24, RM62 and RM84 more frequently observed in low tolerant to Al wheat genotypes. Gluster 3 consist of 7 variables and two of them (RM19 and RM54) are determined with low distances from the respective cluster centre (Table 2c).

The presented analysis is the reason to suggest that the base group of gliadins which could be associated with high tolerance to Al is composed of three strong developed bands describing 1BL/1RS translocation.

Conclusions

Wheat genotypes with 1BL/1RS translocations are found to be more tolerant to aluminium phytotoxicity. The presence of bands in gliadin patterns produced by Sec-1 locus is associated with high tolerance to Al. Although the results need to be confirmed in additional genetic backgrounds, breeders interested to increase the yield and stress resistance by incorporation of 1BL/1RS translocation could consider using it as additional opportunity for crop performance under subsoil acidity.

Acknowledgement

This work was supported in part by a grant CC518/95 from The National Research Found at The Ministry for Education and Science in Bulgaria

References

Barchelo, J., Ch. Poshenrieder, M. D. Vazquez and B. Gunce, 1996. Aluminium phytotoxicity. *Fertilizer Research*, **43:** 217-223.

Clark, R. B., 1982. Plant response to mineral element toxicity and deficiency. In: Christiansen, M. N. and C. F. Lewis (eds) Breeding Plants for Less Favourable Environments, pp. 71-142. *John Wiley & Sons*, New York.

Lawrence, G. J. and K. W. Sheperd, 1981. Chromosomal location of genes controlling seed proteins in species related to wheat. *Theoretical and Applied Genetics*, **59:** 25-31.

MacIntosh, R. A., 1983. A catalogue of gene symbols for wheat. In: S. Sacamoto (ed) *Proc. VI Intl. Wheat Genetic Symp.* Kyoto, Japan, pp. 1197-11255.

Massot, N., M. Llugani, Ch. Poschenrieder and J. Barcelo, 1999. Callose production as indicator of aluminium toxicity in bean cultivars. *Journal of Plant Nutrition*, **22** (1): 1-10.

Rajaram, S., E. Mann, G. Ortiz-Ferrara and A. Mujeeb-Kazi, 1983. Adaptation, stability and high potential of certain 1B/1R CIMMYT wheats. In: (ed. S. Sakamoto) *Proc. VI Intl. Wheat Genetics Symp.* Kyoto, Japan, pp. 613-621.

Shainberg, I., M. E. Summer, W. P. Miller, M. P. W. Farina, M. A. Pavan and M. V. Fey, 1989. Use of gypsum on soil: a review. In: Advances in Soil Science (ed. B. A. Stewart) 9: 1-11. *Springer Verlag*, New York.

Stoyanova, S. D., 1991. Genetic shifts and variation of gliadins induced by seed ageing. *Seed Science and Technology*, **19:** 363-371.

Stoyanova, S. D., D. B. Boyadjieva and Ch. Ch. Phylipov, 2000. 1BL/1RS translocation in wheat germplasm in relation to aluminium tolerance. *Bulgarian Journal of Agricultural Science*, **6** (2): 155-160.

Tzvetkov, K. S., 1994. Tolerance of triticale cv. Vihren (2n = 6X = 42) to the toxicity of aluminium. *Cereal Research Comonnication*, **22** (3): 257-263.

Vazquez, M. D., Ch. Poschenrieder, I. Corrales and J. Barcelo, 1999. Change in apoplastic aluminium during the initial growth response to aluminium by root of a tolerant maize variety. *Plant Physiology*, **119:** 435-444.

William, M. D. H. M., O. Riera-Lizarazu and A. Mujeeb-Kazi, 1992. A combination of protein electrophoretic techniques for the detection of 1B. 1B/1R heterozygotes in *Triticum aestivum* L. *Journal of Genetics and Breeding*, **46:** 137-142.

Zeller, F. J., 1973. 1B/1R substitution and translocations. In: E. R. Sears and L. M. S. Sears eds., *Proc. IV Intl. Wheat Genetics Symp.*, University of Missouri, Columbia, Missouri, pp. 209-221.

Received February, 10, 2001; accepted May, 11, 2001.

Bulgarian Journal of Agricultural Science, 7 (2001), 255-260
National Centre for Agrarian Sciences

Gliadin Analysis for Identification of 1BL/1RS Wheat-Rye Translocations and for Assessment of Protein Patterns in Aluminium Tolerant Genotypes

S. D. STOYANOVA, D. B. BOYAJIEVA and Ch. Ch. PHYLIPOV
Institute for Plant Introduction and Genetic Resources, BG-4122 Sadovo, Bulgaria

Abstract

STOYANOVA, S. D., D. B. BOYADJIEVA and Ch. Ch. PHYLIPOV, 2001. Gliadin analysis for identification of 1BL/1RS wheat-rye translocations and for assessment of protein patterns in aluminium tolerant genotypes. *Bulg. J. Agric. Sci.*, 7: 255-260

Wheat germplasm of different origin (58 accessions) were evaluated by acid gliadin electrophoresis to describe genotypes possessing 1BL/1RS translocation. Wheat cv. Marquis was used to detect the relative mobility (RM) of gladin components. As a marked for 1RS group of bands in the area of w-gliadins was used wheat cv. Kavkaz. Aluminium tolerance was described both for 1BL/1RS translocated wheat and for non-translocated. The effect of aluminium was presented by root growth indexes at four Al-media (0 mM, 0.18 mM, 0.36 mM, 0.72 mM and 1.4 mM). Cluster analysis of gliadin components was carried out to specify the association between gliadin patterns and tolerance to toxic aluminium. 1BL/1RS translocated wheats were found to be more tolerant to Al-toxicity.

Key words: wheat, 1BL/1RS translocation, gliadin, electrophoresis, aluminium tolerance

Introduction

Aluminium toxicity is considered the most important metal toxicity problem on acid soils. As nearly half of the non irrigated arable lands in the world are acid soils, Al toxicity is a severe problem for crop production (Clark, 1982; Barchelo et al., 1996). One of the proposed strategies for crop performance under subsoil acidity is to stimulate the breeding of Al-tolerant genotypes (Shainberg et al., 1996).

Usually the firs observed symptoms of Al injury is an inhibition of root growth as a result of inhibited root elongation (Barchelo et al.,1996; Vazquez et al., 1999). Although much work has been done on the mechanism of Al-toxicity, the differences between species in their ability to resist of tolerance the Al-present in soil is still a point of discussion (Massot et al., 1999). Rye is known as more tolerant than wheat to aluminium toxicity because could be successfully growth in acid soil. High tolerance to aluminium toxicity is presented for triticale cv. Vihren which might due to the rye genes (Tzvetkov, 1994). It is reported that the developed in CIMMYT line 'Alondra S' bearing 1BL/1RS wheat-rye translocation along with the other characters possesses a moderate tolerance to aluminium (Rajaram et al., 1983).

Bulgarian Journal of Agricultural Science, 7 (2001), 261-270
National Centre for Agrarian Sciences

Effect of Imidacloprid on the Tobacco Aphid (*Myzus nicotianae* Blackman) and the Peach Aphid (*Myzus persicae* Sulz.) – Vectors of Virus Diseases

O. KARADJOVA[1], D. HRISTOVA[1] and G. ADAM[2]

[1] *Plant Protection Institute, BG-2230 Kostinbrod, Bulgaria*
[2] *University of Hamburg, Germany*

Abstract

KARADJOVA, O., D. HRISTOVA and G. ADAM, 2001. Effect of imidacloprid on the tobacco aphid (*Myzus nicotianae* Blackman) and the peach aphid (*Myzus persicae* Sulz.) – vectors of virus diseases. *Bulg. J. Agric. Sci.*, 7: 261-270

Cucumber mosaic virus (CMV) and Potato virus Y (PVY) are economically important plant viruses causing diseases of many vegetables and field crops in Bulgaria. Both viruses are non-persistently transmitted by aphids. The green peach-potato aphid – *Myzus persicae* Sulz. and the tobacco aphid *Myzus nicotianae* Blackman are among the most important vectors of CMV and PVY. The transmission efficiency of CMV U^{o} and PVY^{n} by *M. nicotianae* and *M. persicae* was investigated. The results from ACP-ELISA and DAS-ELISA showed that *M. nicotianae* transmit CMV U^{o} and PVY^{n} more efficiently than *M. persicae*. Average CMV transmission rate for *M. nicotianae* was 77.9% ± 11.07, whereas this of *M. persicae* was 57.36% ± 8.06. The tobacco aphid transmitted PVY^{n} almost two times more efficiently than the green peach aphid (75.82% versus 44.21%). It was also investigated the virus incidence after treatment with imidacloprid and acetamiprid under field and laboratory conditions. Imidacloprid is more effective against *M. nicotianae* and *M. persicae* than acetamiprid. Both tested insecticides could not prevent the virus infection in the tobacco field and to kill viruliferous aphids before they induce damage The laboratory test revealed that *M. nicotianae* was less susceptible to the tested insecticides than *M. persicae*.

Key words: peach aphid, virus deseases, imidacloprid, tobacco aphid

Introduction

Cucumber mosaic virus (CMV) and Potato virus Y (PVY) are economically important plant viruses causing diseases of many vegetables and field crops in Bulgaria.

CMV (genus *Cucumovirus*) has an extremely broad host range, which exceeds 775 different plant species in more than 85 distinct botanical families (Douine et al., 1979; Raccah, 1986; Ng and Perry, 1999). CMV is wide distributed virus in Bulgaria and strains belonging to subgroup I and to subgroup II were identified. It was established that strains from subgroup I are predominant (Hristova et al., 2000b). An epidemic of CMV on tobacco was reported in 1986 in Northeastern Bulgaria.