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Variation of Alfalfa Mosaic Virus (AMV) symptoms in lucerne germplasm

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SUMMARY – *Alfalfa mosaic virus* (AMV) is a widespread pathogen on lucerne. In the third year of growth, the percentage of plants with symptoms caused by AMV under natural conditions was recorded on 14 genotypes belonging to different taxonomic groups within the *Medicago sativa* complex (subspp. *sativa*, *falcata*, *varia*, and hybrids among different taxa), as also indicated by their different flower colour. The genotypes, all characterised by deep-set crown, were also morphologically diversified and categorised into four models, varying in growth habit and vigour. Significant differences were found among models and genotypes; the two most erect and vigorous models had, on average, higher percentage of plants with symptoms (>80%) than the very prostrate and rhizomatous model (<15%). Genotypes ranged between 1.4% and 97.6% of plants with symptoms. Such a variation appeared to be related with differences in flower colour and, hence, taxonomic origin of the germplasm: the mean frequency of plants with symptoms in purple-flowered genotypes (typically of subsp. *sativa*) was 70.7% versus 24.6% in genotypes with other flower colours. The two genotypes with the highest percentage (>95%) and the two with the lowest (<10%) were again evaluated in the next year and confirmed the sharp differences in symptoms already observed. In addition, a strong relationship was evidenced between the presence of plants with symptoms in a plot and aphid infestation in the same plot.

Key words: Aphid, *alfalfa mosaic virus* (AMV), germplasm, lucerne, *Medicago sativa* L. complex.

RESUME – *"Variabilité des symptômes du virus de la mosaïque de la luzerne dans le germoplasme de cette espèce". Le virus de la mosaïque de la luzerne (Alfalfa mosaic virus = AMV) est un pathogène très répandu de cette culture. Pendant la troisième année de croissance, le pourcentage de plantes avec des symptômes causés par l'AMV a été enregistré sur 14 génotypes appartenant à des groupes taxonomiques différents du complexe* Medicago sativa *(subspp.* sativa, falcata*,* varia*, et hybrides parmi des taxa différents), comme indiqué aussi par leurs différentes couleurs des fleurs. Les génotypes, tous caractérisés par une couronne profonde, étaient aussi diversifiés morphologiquement et catégorisés dans quatre modèles à port et vigueur différente. L'on a trouvé des différences significatives parmi les modèles et les génotypes; les deux modèles les plus dressés et vigoureux avaient, en moyenne, un pourcentage de plantes avec des symptômes (>80%) plus élevé que celui du modèle très rampant et rhizomateux (<15%). Les génotypes montraient entre 1,4% et 97,6% de plantes avec des symptômes. Cette variabilité a paru en relation avec les différentes couleurs des fleurs et, par conséquent, l'origine taxonomique du germoplasme: la fréquence moyenne dans les génotypes à fleurs violettes (typiques de la subsp.* sativa*) était de 70,7% vis-à-vis de 24,6% dans les génotypes avec d'autres couleurs des fleurs. Les deux génotypes avec le pourcentage le plus haut (>95%) et les deux avec le plus bas (<10%) ont été évalués encore pendant l'année suivante et ils ont confirmé la grande différence de symptômes déjà remarquée. De plus, l'on a mis en évidence une forte relation entre la présence de plantes avec des symptômes dans une parcelle et l'infestation de pucerons sur la même.*

Mots-clés : *Pucerons, complexe* Medicago sativa *L., germoplasme, luzerne, virus de la mosaïque de la luzerne (Alfalfa mosaic virus = AMV).*

Introduction

Alfalfa mosaic virus (AMV) is a widespread pathogen responsible of attacks on over 400 plant species belonging to more than 50 families (Hiruki and Hampton, 1990). In lucerne (*Medicago sativa* L. complex), the symptoms generally appear in spring, mainly on the regrowth following the first cut. Because of the wide array of existing virus strains, the plant response can vary considerably, including foliar mottle, stunted growth or, even, death; one common symptom is leaf and petiole distortion (Frosheiser, 1969; Hiruki and Hampton, 1990). Virus particles can be transmitted in plant saps, pollen or seed. The pea aphid, *Acyrthosiphon pisum* (Harris), is considered one of the main vectors of AMV, although other aphid species can as well transmit the virus (Leath *et al*., 1988). Long-distance spread and establishment of primary infections are attributable to transmission through seed, whereas short-distance spread and re-

infections likely result from transmission by aphids, pollen or agricultural machinery (Hiruki and Hampton, 1990). Losses in lucerne forage yield, up to 30%, and reduced winter survival caused by AMV were reported in North America (Frosheiser, 1969; Leath *et al.*, 1988). The virosis is considered a serious disease in Southern and Eastern Europe (Raynal, 1986; Delgado Enguita and Luna Calvo, 1992). Leath *et al.* (1988) and Hiruki and Hampton (1990) reported of lucerne genotypes resistant to one or more virus strains but of none resistant to all known strains, which makes it difficult to select resistant cultivars. Frosheiser (1969) and Tavoletti *et al.* (1992) found tolerant genotypes in susceptible cultivars, that is, plants that did not show any symptom, although being infected by the virus.

The current study was undertaken on a diversified set of lucerne germplasm to assess the occurrence of AMV symptoms under natural conditions in Northern Italy.

Materials and methods

The trial included 14 lucerne genotypes belonging to different taxonomic groups within the species complex and, therefore, characterised by purple, yellow or variegated flower colour according to their genetic origin (Table 1).

Table 1. Mean frequency of plants with symptoms by AMV at the third year of growth in different genotypes and flower colour classes examined in the current study[†]

Genotype	Taxonomic attribution	Morphological model	Flower colour	Plants with virus symptoms (%)
346/1	Subsp. *sativa*	D2	Purple	97.6 a
300/16	Subsp. *sativa*	D4	Purple	96.8 a
300/12	Subsp. *sativa*	D3 - hv	Purple	82.6 b
108/1	Subsp. *sativa*	D4	Purple	77.2 b
11/5	Artificial hybrid	D2	Purple	71.8 b
275/19	Subsp. s*ativa*	D2	Purple	47.6 c
253/17	Subsp. *varia*	D3 - lv	Variegated	45.4 c
316/4	Artificial hybrid	D3 - lv	Variegated	36.5 c
287/9	Subsp. *varia*	D3 - hv	Purple	28.7 cd
61/13	Subsp. *varia*	D1	Variegated	27.1 cd
91/3	Artificial hybrid	D3 - hv	Variegated	26.5 cd
14/8	Subsp. *varia*	D3 - lv	Variegated	26.3 cd
283/14	Subsp. *falcata*	D1	Yellow	9.6 d
37/1	Subsp. *falcata*	D1	Variegated	1.4 d
			Mean purple	70.7 A
			Mean variegated + yellow	24.6 B

[†]The statistical analyses refer to the angular transformation [arcsin $\sqrt{(x/100 + 0.5)}$] of the percentages even though, for sake of clarity, the original values are reported in the Table.

[a,b,c,d]Among genotypes, means followed by the same letter are not different at $P \leq 0.05$ according to Duncan's multiple range test.

[A,B]Means of flower colour classes different at $P \leq 0.05$ according to analysis of variance.

On the basis of the growth habit and vigour of the aerial part, the genotypes were categorised into four morphological models according to Piano *et al.* (1996). The D1 model was prostrate and very rhizomatous, while the D2 and D4 models were substantially erect and rather vigorous; the D3 was somewhat intermediate in habit and was split into two subgroups of lower- (lv) and higher-vigour (hv) genotypes (Table 1). The experiment was a randomised complete block design with four replications, each including 3 plots of 9 spaced plants for each genotype. During the third year, the frequency of plants showing AMV symptoms was recorded in early June on the regrowth of the first cut. By analysis of variance (ANOVA), after angular transformation of the original data, differences among genotypes, among

morphological models and between flower colour classes (purple vs. other colours) were tested. On the basis of this screening, attention was given to two pairs of genotypes with contrasting behaviour, viz. '346/1' and '300/16' (belonging to subsp. *sativa*), and '283/14' and '37/1' (belonging to subsp. *falcata*), which were again examined in the following year. At the date of the first cut (early May) a score was attributed to each plant for the presence and severity of symptoms, on a scale from 1 (absent) to 4 (present on the whole plant) similar to the score adopted by Tavoletti *et al.* (1992); on the subsequent regrowth (early June), the percentage of plants showing symptoms was also recorded, together with the presence/absence of aphids (*Aphis fabae* Scop.) on the whole plot. Differences among genotypes for symptom score and percentage were tested by ANOVA. The stochastic independence between the presence of plants with symptoms in a plot and the aphid infestation in the same plot was tested by Fisher's exact test.

Results and discussion

The ANOVA revealed significant ($P \leq 0.001$) differences for the frequency of plants with symptoms both among genotypes and between flower colour classes (data not shown). Mean values of genotypes and colour classes are reported in Table 1. The data indicate that great variation of response to AMV was present in the examined germplasm, which represented a large genetic base, with genotype values ranging from 1.4% to 97.6% of plants showing symptoms. Such a variation was clearly related to differences in flower colour, as evidenced by the fact that six out of seven purple-flowered genotypes had the highest frequency of symptomatic plants. As a result, the mean frequency was 70.7% in genotypes with purple flowers, and 24.6% in genotypes with other flower colours (Table 1). On the whole, the germplasm belonging to subsp. *sativa* appeared, therefore, more prone to the virus than that of other subspecies.

Differences among morphological models, which also bear a certain taxonomic relevance (Piano *et al.*, 1996), were significant ($P \leq 0.001$) according to ANOVA (data not shown). The model mean values were: D4 = 87.0% and D2 = 84.9% > D3-hv = 44.9% and D3-lv = 36.1% > D1 = 14.7%, the equivalence or superiority of means being indicated according to the Duncan's multiple range test. The two substantially erect models (D4 and D2) had higher frequency of plants with symptoms (>80%) than the semi-erect and rhizomatous D3 model (in both vigour variants) and the prostrate, very rhizomatous D1 model. This last model is also characterised by the longest cold-season dormancy (Piano *et al.*, 1996) and, in that, the present results agree with those by Delgado Enguita and Luna Calvo (1992) who found that the germplasm with the longest winter dormancy was the less affected by the virus.

The ANOVA of the data recorded in the subsequent year on four genotypes showed significant ($P \leq 0.001$) differences for symptom score and frequency (data not reported). The genotypes '346/1' and '300/16' had a mean score of 3.9 and a frequency of symptomatic plants of 95.2%, significantly ($P \leq 0.05$) higher than those of the genotypes '283/14', and '37/1' (1.1 and 3.5%, respectively), according to Duncan's multiple range test. The consistency of behaviour in the two years of evaluation shown by the two pairs of genotypes suggests an actual genotypic implication on AMV symptom displaying. In particular, the two subsp. *falcata* genotypes '283/14', and '37/1' could be tolerant genotypes as expressed by Frosheiser (1969) and Tavoletti *et al.* (1992), that is, genotypes hosting the virus but not showing symptoms (except for a very few plants). However, it cannot be excluded *a priori* the presence of resistance, or avoidance, to the virus infection. As a matter of fact, a remarkable relationship has emerged between the presence of plants with symptoms and the aphid infestation or, viceversa, between the absence of aphids in the plot and the lack of symptomatic plants. The independence between the two traits can indeed be rejected at $P \leq 0.001$ by both Pearson's chi-square and Fisher's exact tests, and the value of the coefficient of non-parametric correlation indicates a positive relationship between them (Table 2). The presence of endogenous compounds deterrent to insects, such as for instance saponins (Julier *et al.*, 1996), or, more likely, a mechanical hindrance by leaf cell morphology to aphid nutrition (Alliot, 1990; Girousse and Bournoville, 1994), could be advocated for a lower incidence of AMV in the subsp. *falcata*.

Table 2. Test of stochastic independence between the presence in the plot of plants with symptoms by AMV and the presence of aphid in the same plot

	Number of plots *without* aphid infestation	Number of plots *with* aphid infestation
Number of plots *without* plants showing AMV symptoms	22 (% row : 100.0 (% column : 66.7	0 0.0) 0.0)
Number of plots *with* plants showing AMV symptoms	11 (% row : 42.3 (% column : 33.3	15 57.7) 100.0)

Test	DF	Value	Probability
Pearson^2	1	17.8	< 0.001
Fisher's exact test (2-tail)	1		< 0.001
Coefficient	1	0.61	

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