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Relationship between cyanogenic compounds in seeds, leaves and roots of sweet and bitter kernelled almonds

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SUMMARY – The relationship between the resistance to capnode (*Capnodis tenebrionis* L.) in almond (*Prunus dulcis* Miller) and the presence of some cyanogenic compounds in roots has been pointed out. However, some aspects such as the relationship between the content of cyanogenic compounds in the different parts of the plant remain unclear. In this work the content of these compounds in kernels, leaves and roots of 5 sweet kernelled, 5 slight bitter kernelled and 5 bitter kernelled almond trees was determined. The results show a good correspondence between the content of cyanogenic compounds in kernels and roots. Given that inheritance of kernel bitterness is known to be a monogenic trait, these results open new possibilities for the breeding of new cultivars with a high content of cyanogenic compounds in roots, probably resistant to capnode.

Key words: Almond, *Prunus dulcis* Miller, bitterness, cyanogenic compounds, amygdalin, prunasin, capnode, *Capnodis tenebrionis* L.

RESUME – *"Relation entre composés cyanogènes dans les semences, les feuilles et les racines d'amandes douces et amères". La relation entre la résistance à la capnode (*Capnodis tenebrionis *L.) chez l'amandier (*Prunus dulcis *Miller) et la présence de certains composés cyanogènes dans les racines a été remarquée. Cependant, certains aspects tels que la relation entre la teneur en composés cyanogènes dans les différentes parties de la plante restent sans éclaircir. Dans ce travail a été déterminée la teneur en ces composés dans les amandons, les feuilles et les racines de cinq amandiers à fruits doux, 5 à fruits légèrement amers et 5 à fruits amers. Les résultats montrent une bonne correspondance entre la teneur en composés cyanogènes dans les amandons et les racines. Etant donné que l'hérédité de l'amertume du fruit est reconnue être un caractère monogénique, ces résultats ouvrent de nouvelles possibilités pour la sélection de nouveaux cultivars ayant une forte teneur en composés cyanogènes dans les racines, probablement résistants à la capnode.*

Most-clés : *Amandier,* Prunus dulcis *Miller, amertume, composés cyanogènes, amygdaline, prunasine, capnode,* Capnodis tenebrionis *L.*

Introduction

The capnode (*Capnodis tenebrionis* L.) is a coleopteran found in the Mediterranean countries, although it has been described in other countries of Europe and Central Asia (Malagón, 1989). It affects all the *Rosaceae* both wild and cultivated, including the fruit trees of the genus *Prunus* (apricot, peach, plum, cherry and almond trees), and it has also been detected in other fruit trees such as quince, pear, apple, hazel and fig (Malagón, 1989). The most important damage is produced by the grubs that get into the roots, destroy the vascular tissues and frequently cause the death of the tree (Garrido, 1984).

Some research reports show a relationship between the resistance to capnode and the presence of some cyanogenic glycosides in the roots, owing to the fact that the grub does not penetrate them or dies immediately after entering (Malagón and Garrido, 1990; Usai and D'hallewin, 1990; Mulas, 1994). These compounds are found in variable quantities both in the seeds (mainly amygdalin) and in the vegetative parts (mainly prunasin) of several *Prunus* species.

However, the relationship between these compounds in seeds and roots has not been determined. If there was close relationship, it would be simple to obtain almond trees with high concentrations of cyanogenic compounds in the roots, since the control of the bitter flavour of the seed is well-known (Dicenta and García, 1993).

The objective of this work is to determine the relationship among cyanogenic compounds in kernels, roots and leaves of a tree to design strategies of breeding almonds with high quantities of cyanogenic compounds in their roots, probably conferring resistance to *Capnodis tenebrionis*.

Material and methods

15 descendants of a cross between Garrigues and Tuono almond cultivars were studied. Five are sweet, 5 slight bitter, and 5 bitter kernelled. Descendants were grown on their own roots.

During spring, leaf and root samples were taken from the 15 genotypes. The seeds, of a previous crop, were collected in the mature state. Seeds, roots and leaves were lyophilised and prepared for analysis of total cyanide. At least two repetitions per sample were analysed. Total cyanide measurement involves:

(i) Release of hydrogen cyanide by enzymatic hydrolysis (with beta-glucosidasa from sweet almonds), at pH 5.5 and 35ºC for 24 h, with collection in NaOH solution, following the procedure described by Williams (1979).

(ii) Gravimetric titration with $AgNO_3$ standard solution and dimethylaminobencilidenrhodanine indicator (Williams, 1979), for samples containing more than 20 mg cyanide/100 g sample, or spectrophotometric determination at 580 nm, following derivatization with barbituric acid in pyridine (Asmus, 1953), for samples with a lower cyanide content.

The data (mg CN/100 g) were analysed by ANOVA to determine if the cyanide content in roots and leaves depended on the genotype and on the flavour of the seed (sweet, bitter, slight bitter). For analysing the means, the Duncan's test was used. Pearson's correlation coefficient for cyanide content of leaves, roots and seeds was calculated.

Results

The analysis of variance detected significant differences among the 15 genotypes for the cyanide content of seeds, roots and leaves. In Fig. 1 the means for the 15 studied genotypes are represented.

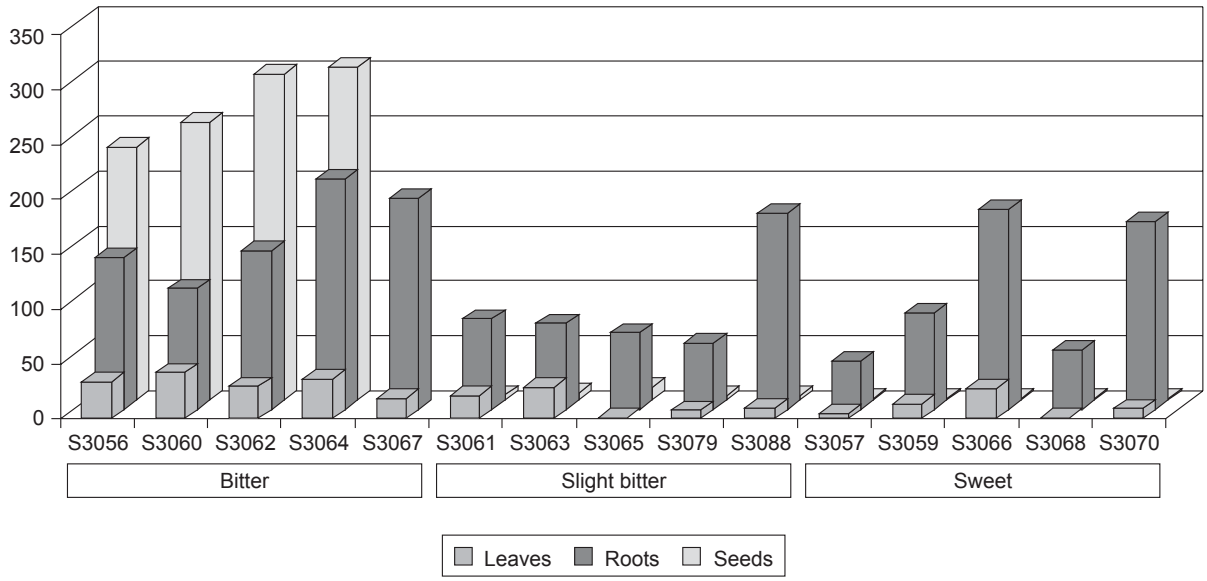

Fig. 1. Mean cyanide content (mg CN/100 g) in leaves, roots and seeds of 5 sweet, 5 slight bitter and 5 bitter kernelled almonds.

In seeds, the values of cyanide of the bitter kernelled almonds was much higher than those of non-bitter kernelled genotypes (slight bitter and sweet).

In the case of the roots, the bitter kernelled genotypes exhibited high cyanide concentrations again, the levels being lower in roots of non-bitter kernelled. Some exceptions like S3088 (slight bitter), S3066 and S3070 (sweet), exhibited in their roots similar or even higher cyanide levels than some bitter genotypes.

In the leaves, the cyanide values were much smaller than in seeds and in roots. Again, the bitter genotypes gave superior values of cyanide, except for S3061 and S3063 (slight bitter) and S3066 (sweet).

In Fig. 2 the 15 genotypes have been regrouped into sweet, slight bitter and bitter kernelled. The analysis of variance detected significant differences for the cyanide content of seeds, roots and leaves between groups. Duncan's test distinguished two groups with respect to the three organs: bitter and non-bitter kernelled (sweet + slight bitter).

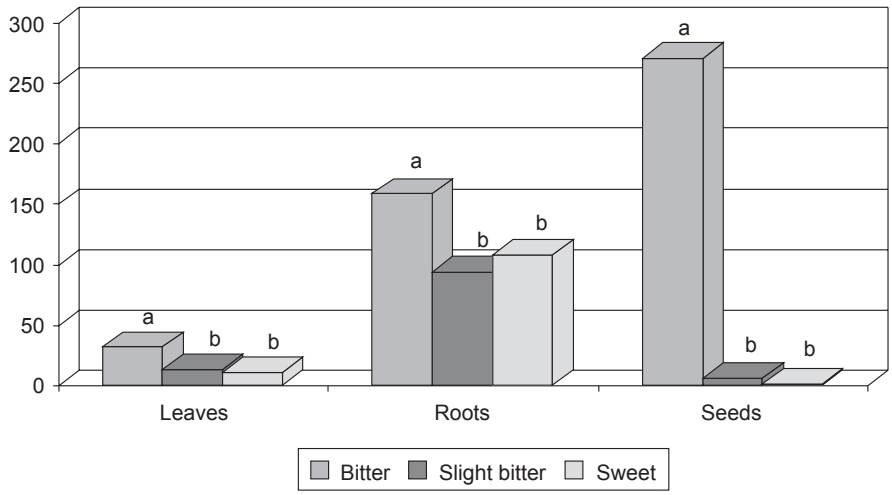

Fig. 2. Mean cyanide content (mg CN/100 g) in seeds, roots and leaves of the sweet, slight bitter and bitter kernelled almonds, and Duncan's test. The bars with the same letter show no significant differences.

The mean values of cyanide in the bitter individuals were higher than those of non-bitter in seeds, roots and leaves. The slight bitter and sweet kernelled almonds had a very similar behaviour, with their cyanide contents being very low in seeds and leaves and intermediates in roots.

Regarding the relationship of the cyanide content in the studied organs, Pearson's correlation coefficient was 0.45 in the case of roots-leaves and roots-seeds, and 0.74 for leaves-seeds. In the case of the bitter individuals, the correlation coefficient between roots and seeds was 0.68.

Discussion

The data seem to indicate that, in general, bitter kernelled almond have more cyanide in the leaves and in the roots, although some non-bitter ones also showed this characteristic.

The important differences in the content of cyanogenic compounds in bitter and non-bitter seeds was also observed in other work (Usai and D'hallewin, 1990; Dicenta *et al*., 1999). Nevertheless, this close relationship may not be so strict in the case of other parts of the plant (leaves and roots).

Usai and D'hallewin (1990) found no correlation between amygdalin and prunasin content in seeds of one cultivar and the prunasin content in shoots and roots of seedlings produced by open pollination of this cultivar, which was expected because of variability generated by open pollination.

Mulas (1994) obtained a correlation coefficient of 0.97 between prunasin concentration in shoots and roots of several seedlings of *Prunus* species, sweet and bitter almond included, but he did not study the seeds.

Since amygdalin (in seeds) is a diglucoside formed from the monoglucoside prunasin (vegetative parts) by the action of the gluclosyltransferase (Frehner *et al.,* 1990), the relationship between these two compounds would allow the creation of almond trees with high quantities of cyanogenic compounds in the roots, by crossing bitter almonds or even by their self-pollination.

The prunasin production in the vegetative part may be a quantitative trait and its transformation in amygdalin into the seed, a monogenic trait controlling this reaction. So, the bitter kernelled genotypes must to have prunasin in their vegetative part, while sweet kernelled may or may not have prunasin. This would explain the high prunasin levels in roots in all bitter kernelled genotypes, and the high or low levels in those of sweet kernelled genotypes. In any case, it is clear that bitter kernelled almonds have high levels of cyanogenic compounds in their roots.

From the breeding point of view, the research into bitter kernelled rootstocks complements other objectives, such as the increase of the productivity (Spiegel-Roy and Weinbaum, 1985) or the resistance to nematode (Kochba and Spiegel-Roy, 1976).

Furthermore, Atocha, Desmayo Largueta and Garrigues, the main Spanish cultivars used as sources of seedling rootstocks (Simard *et al.,* 1997), could generate bitter kernelled seedlings, since they are heterozygous for bitterness. Of course vegetative multiplication of the genotypes selected would be desirable.

Finally, the specific analysis of the cyanogenic compounds (amygdalin and prunasin) in controlled conditions (plants in pots) could contribute to explain the relationship between the content of these compounds in seeds and the vegetative part.

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