Evaluation of almond flower tolerance to frosts by chlorophyll fluorescence

O. Kodad*,, R. Socias i Company* and F. Morales*****

* Unidad de Fruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA)
Av. Montañana 930, 50059 Zaragoza (Spain)
** Present address: INRA Meknes BP 578, Meknes (Morocco)
*** Estación Experimental de Aula Dei, CSIC, Apartado 202, 50080 Zaragoza (Spain)
rsocias@aragon.es

Abstract. Most almond cultivars are susceptible to negative temperatures, a limiting factor for almond expansion to regions with risks of spring frosts. Flower and fruitlet tolerance to frosts has only been studied so far by observing the morphological damages produced by low temperatures. Thus, our objective was the evaluation of chlorophyll fluorescence (CF) to estimate the tolerance of 12 commercial almond cultivars of different origin to low temperatures. Flowers were maintained for 24 hours at different temperatures (0º, -1º, -2º and -3ºC), after which CF was measured. In general, the variable fluorescence (Fv) and the ratio Fv/Fm decreased with time in all genotypes, although the rate of decrease depended on the genotype. The decrease of these parameters was slower in the cultivars tolerant to low temperatures, whereas the decrease was linear or/and sigmoid in the susceptible cultivars. In general, the classification of genotypes with this technique according to their frost tolerance level agreed with the published references. These results point out that chlorophyll fluorescence is a promising technique (fast, quantitative, easy and non-destructive) to ascertain the tolerance of almond genotypes to frosts independently of their blooming time.

Keywords. Almond – Flower – Tolerance – Frosts – Chlorophyll fluorescence – Breeding.

Evaluation de la tolérance des fleurs d'amandier à la gelée par fluorescence de la chlorophylle

Résumé. *La plupart des variétés d'amandier sont sensibles aux gelées, ce qui constitue un facteur déterminant dans la propagation et l'expansion de sa culture aux régions à haut risque de gelées printanières. De nos jours, la tolérance des fleurs et des petits fruits aux gelées a été évaluée en observant visuellement les dégâts morphologiques causés par les gelées. Notre objectif est l'évaluation de l'usage de la fluorescence de la chlorophylle (CF) pour estimer la tolérance de 12 cultivars commerciaux d'amandier de différentes provenances aux faibles températures. Avant de procéder aux mesures de CF, les fleurs ont été soumises pendant 24 h à différentes températures (0º, -1º, -2º y -3ºC). En général la fluorescence variable (Fv) et le ratio Fv/Fm ont diminué avec la température chez tous les génotypes, malgré que la vitesse de réduction était variable en fonction du génotype. Le taux de réduction de ces paramètres est plus lent chez les cultivars qui tolèrent les basses températures, alors que la réduction est quadratique ou linéaire chez les génotypes sensibles. En général, la classification des génotypes avec cette technique en fonction de leur tolérance aux gelées est en concordance avec les résultats publiés. Ces résultats indiquent que la fluorescence de la chlorophylle est une technique prometteuse (rapide, quantitative, facile et non destructive) pour évaluer la tolérance des génotypes d'amandier aux gelées printanières indépendamment de leur époque de floraison.*

Mots-clés. *Amandier – Fleur – Tolérance – Gelées – Fluorescence de la chlorophylle – Amélioration.*

I – Introduction

Spring frost injury is a major limiting factor in the production and distribution of horticultural crops. Almond (*Prunus amygdalus*, Batsch) is an early blooming species, thus susceptible to spring frosts. The expansion of the culture into inland Mediterranean areas, where the occurrence of spring frosts is common and coincident with bloom of most almond cultivars, increased the risk of reducing or even nullifying yield (Kodad and Socias i Company, 2005;

Socias i Company *et al*., 1999). Thus, almond breeding aimed to obtain late blooming cultivars (Kester and Assay, 1975). Nevertheless, this solution is not always enough to overcome frost damages, and frost hardness is also considered a selection objective in a breeding programme (Socias i Company *et al.*, 1998).

The evaluation of frost tolerance of almond flowers has shown the presence of a high genotypic variability in response to very low temperatures and to frost stress (Felipe, 1988; Kodad and Socias i Company 2005; Snyder and Connell, 1995). The possibility of incorporating this trait in an almond breeding programme requires the use of a fast, quantitative and easy technique to screen for tolerance to spring frosts, due to the high number of genotype to be evaluated. This evaluation has been based on traditional methods, such as by visual assessment of frost damages on flowers and fruits (Büyükyilmaz and Kester, 1976; Kodad and Socias i Company, 2004) or by evaluating the magnitude of damages on fruit set and yield (Felipe, 1988; Kodad and Socias i Company, 2005). All these methods are time consuming, difficult and dependent on the occurrence of frosts.

Chlorophyll fluorescence (CF) has been used to study plant responses to different kinds of stress (reviewed by Baker and Rosenqvist, 2004), including the evaluation of tolerance of flowers to spring frosts (Khanizadeh and DeEll, 2001). The variable fluorescence (Fv) has been used to evaluate the chilling and freezing tolerance in different plant (Karukstis, 1991). Fv is influenced by temperature and frost stress leads to a significant decrease of CF (Karukstis, 1991; Khanizadeh and DeEll, 2002). As a general rule, susceptible and tolerant cultivars exhibit similar patterns of chlorophyll fluorescence reductions, although the reduction is accelerated with chilling sensitivity (Hakam *et al*., 2000; Karukstis, 1991; Khanizadeh *et al*., 2000; Khanizadeh and DeEll, 2001).

The objective of this study was to evaluate the possibility of using CF measurements for evaluating almond flower tolerance to frost stress.

II – Material and methods

1. Plant material

Twelve commercial almond cultivars (Table 1) were studied. All plant samples were obtained from the Spanish almond germplasm collection located at the CITA, Zaragoza, maintained as living plants grafted on the almond × peach hybrid clonal rootstock INRA GF-677, using the standard management practices (Espiau *et al*., 2002).

2. Chlorophyll fluorescence measurements

CF measurement was performed following a modified method from that described by Khanizadeh and DeEll (2001). Flowers samples, at phenological stage E (Felipe, 1977), were collected randomly around the trees of each cultivar. The detached flowers were placed immediately in plastic bags containing moist filter paper. In the laboratory, the samples were kept in the dark at 25ºC for 2 h to ovoid pre-irradiation by visible light, which can alter the fluorescence measurements. The flowers were exposed to 25ºC for 24 h, 0ºC for 24 h, -1ºC for 24 h, -2ºC for 24 h, and -3ºC for 24 h. Fv (variable fluorescence) was measured following these temperature treatments.

The measurements were done in a dark room without windows, with a single green 40-watt safe light was used providing a low level of illumination during the measurements. Those were made with an PAM 200 fluorometer (Heinz Walz, Effeltrich, Germany), using the Fv/Fm test (method 1 on the fluorometer). Modulation intensity was set at 80 and the detector gain at 40, with the saturation light (35-W halogen lamp) intensity set at 190 for 0.8 s. The Fv was used for data analysis and calculated as Fv = Fm – Fo, where Fo and Fm are the minimal and the maximal

fluorescence, respectively, of a dark adapted sample. Measurements were made on the ovary region of the flowers, which were kept hydrated. The Fv was determined by placing the pre-chilled sensor of the portable fluorometer on the flower ovary and irradiating with red light of photon flux density 20 $mmol \cdot m^{-2} \cdot s^{-1}$.

Table 1. Evolution pf chlorophyll fluorescence in the ovary zone of flower buds of the cultivars studied as related to temperature. Relative value of de Fv/Fm in relation to the normal at 22ºC (%).

Variety	Temperature					Regression
	22ºC	0ºC	-1ºC	-2ºC	-3ºC	
'Bertina'	100	100,2	85,17	70,02	54,97	Q
'Desmayo Largueta'	100	95,39	61,76	59,87	52,04	Q
'Felisia'	100	99,48	95,63	73,01	65,60	Q
'Ferragnès'	100	91,29	90,54	77,15	62,42	Q
'Guara'	100	96,29	93,48	84,21	76,61	Q
'Marcona'	100	92,23	91,44	88,57	80,31	Q/L
'Masbovera'	100	100,1	88,84	85,31	82,02	Q
'Moncayo'	100	84,74	76,23	74,85	73,12	Q/L
'Nonpareil'	100	98,5	93,1	87,16	78,44	Q
'Peerless'	100	89,08	83,20	65,71	57,87	Q
'Mission'	100	93,39	87,98	86,12	74,08	Q
'Tuono'	100	99,66	99,51	86,56	63,74	Q/L

III – Results and discussion

Fv decreased with temperature in all genotypes (Table 1), coinciding with the results reported in strawberry flowers (Khanizadeh and DeEll, 2001), tomato leaves (Brüggermann and Linger, 1994) and roses (Hakam *et al*., 2000). The pattern of reduction of Fv, in our case, was not similar for all cultivars, as the decrease was quadratic or linear depending on the genotype. Khanizadeh and DeEll (2001) reported in strawberry that when the reduction was quadratic, the genotype was from susceptible to tolerant, whereas when the reduction was lineal, the genotype was less tolerant. In our case, almost all genotypes, with the exception of 'Marcona', 'Tuono' and 'Moncayo', showed a quadratic decrease of their Fv, with a turning point at -1 or -2ºC. This means that until these temperatures were reached, the reduction in Fv was not significant. Thus, the flowers of these cultivars would be tolerant at these temperatures.

On the other hand, Hakam *et al.* (2000) reported that the rapid reduction of Fv indicates a real tissue injury and could be used to assess the level of susceptibility of each cultivar to frost stress. 'Nonpareil', 'Peerless' and 'Mission' showed a quadratic decrease in their Fv, thus these cultivars could be considered from susceptible to tolerant to frost stress. However, the rate of decrease showed by 'Nonpareil' was lower than those of 'Peerless' and 'Mission', showing that 'Nonpareil' is more tolerant than the others. The same results were reported by Snyder and Connell (1995) when evaluated almond frost tolerance in controlled conditions.

'Desmayo Largueta' showed a linear reduction with temperature, whereas in 'Marcona' it was quadratic, showing that the first is more susceptible than 'Marcona'. 'Desmayo Largueta' also showed a rapid decrease of Fv, as related to its high susceptibility to very low temperatures and to spring frosts (Felipe, 1988). During the spring of 2005 a frost of -3ºC took place when both cultivars were at the phenological stage D, producing important damages on 'Desmayo Largueta' flower buds, but insignificant damages on 'Marcona' (data not shown), showing that

'Marcona' flower buds must be more tolerant to frosts than those of 'Desmayo Largueta', in agreement with the results obtained with the technique of chlorophyll fluorescence.

However, the decrease of Fv in some cultivars considered as frost tolerant, such as 'Masbovera' (Vargas, unpublished) and 'Nonpareil' (Snyder and Connell, 1995), showed the same pattern of reduction than 'Ferragnès' and 'Marcona' (Fig. 1), considered as susceptible (Miranda *et al*., 2005), suggesting that these cultivars could be considered between tolerant and susceptible. However, the reduction of Fv in function of temperature is slower for 'Masbovera' than for 'Ferragnès' and 'Marcona' (Fig. 1).

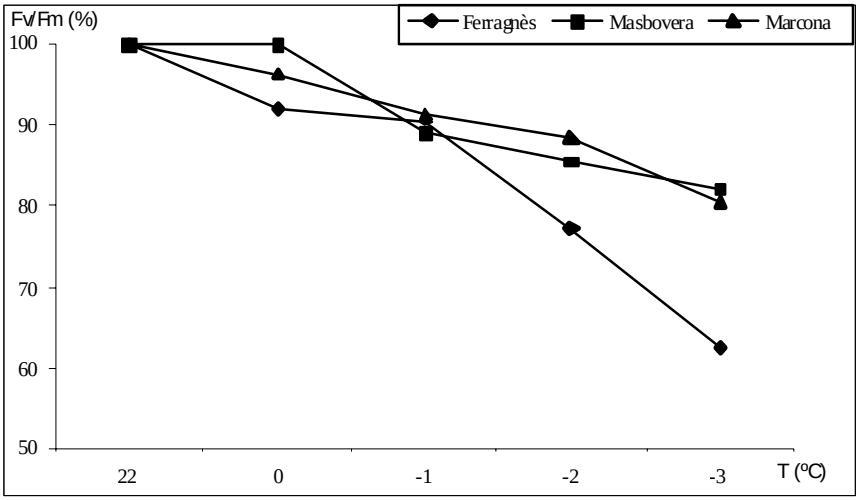

Fig. 1. Evolution of Fv/Fm in 'Marcona', 'Masbovera' and 'Ferragnès'.

Although both the susceptible and tolerant plants show the same pattern of reduction in the emission of fluorescence by chlorophyll, this reduction is sharper in susceptible plants (Bernnan and Jefferies, 1990; Hakam *et al*., 2000). This observation indicates that for rating the susceptibility of different genotypes to frosts it is more important the speed of reduction of the Fv/Fm ratio than the pattern of reduction. With our results it is possible to consider that the pattern of Fv reduction in function of the temperatures proposed by Hakam *et al*. (2000) and Khanizadeh and DeEll (2001) may be adapted to classify frost tolerance in almond.

The results showed that 10 genotypes (Table 1) had a sigmoid response (Q), with a turning point between -1ºC and -2ºC. Consequently, the Fv/Fm reduction was not significant until these temperatures were reached, suggesting that the flower buds of these genotypes must be tolerant at temperatures between 0ºC and -2ºC. Among all genotypes studied, the lower values for chlorophyll fluorescence were measured at temperatures between -2ºC and -3ºC (Table 1), signifying that the flower buds of all genotypes are vulnerable at these temperatures, agreeing with our observations and with previous results (Felipe, 1988; Miranda *et al*., 2005).

IV – Conclusion

The variability in the response to frosts of the different genotypes studied, independently of their blooming season, confirms the existence of sources of frost tolerance in almond. The results of applying the technique of chlorophyll fluorescence for evaluating frost tolerance in this set of cultivars were in agreement with the results obtained in laboratory conditions and with the

published references. These first results suggest the possibility of using chlorophyll fluorescence in a large scale as a promising technique (fast, quantitative and simple) to assess frost tolerance in breeding progenies of a breeding programme. However, this technique is still under evaluation, modification and adaptation to the plant material and to the breeding programme of the Unidad de Fruticultura of the CITA.

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