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Flower Bud Frost Tolerance of Several Italian Apricot Genotypes

R. Viti[1)], S. Bartolini[2)] and L. Andreini[2)]
([1)]Dipartimento di Difesa e Coltivazione delle Specie Legnose "G. Scaramuzzi", Universita di Pisa, Pisa, Italy and [2)]Scuola Superiore 'S. Anna' di Studi Universitari e di Perfezionamento, Pisa, Italy)

Summary

In the Tyrrhenian coastal area, frequent late frosts have occurred during the last ten years. The most severe event happened in 2003, causing a strong reduction in yield, above all in stone fruit species. However, several apricot selections, from different breeding sources, had a considerable yield, as a consequence of high flower and/or young fruit survival. The aim of this research was to evaluate the degree of tolerance to spring frost of ten selections and their own parents. During three subsequent years, the frost sensitivity of flower buds at dormant and swelling phenological stages was determined using the Phenolic Compound Leakage (PCL) test after an artificial frost treatment (−4 °C). When this temperature was applied to flower buds at the dormant stage did not cause significant injuries, as indicated by the low PCL values. At the pre-flowering stages a significant increase in PCL was observed in most genotypes. On the basis of different levels of PCL, the genotypes were classified into three groups in relation to their frost sensitivity: i) very frost-tolerant; ii) frost-tolerant and iii) frost-sensitive. Within the very frost-tolerant group, the selections 7C 20/3, 11/48/7 and 11/19/2, were the best genotypes confirming a good yield performance recorded after the severe spring frost of 2003.

Key words. artificial frost treatment – floral anomalies – flower bud survival – fruit set – phenolic compound leakage – *Prunus armeniaca* L. – spring frost

Introduction

Apricot (*Prunus armeniaca* L.) is a widespread fruit species in the Mediterranean. It is characterized by an early blooming period, at the end of winter when freezing temperatures can still occur. In temperate climates, spring frost is an important environmental factor that limits apricot yields, as a consequence of strong injuries to buds, flowers and developing fruits (Byrne 1986; Gunes 2006). During the autumn-winter period, when apricot flower buds are in an endodormancy status, a high tolerance to frosts has been recorded (Bartolini et al. 2006a). At the beginning of bud swelling, during the ecodormancy release (end of winter-beginning of spring) frost tolerance decreases concomitantly with the reactivation of bud growth (Layne and Gadsby 1995; Hevett 1996; Szalay et al. 2006). In this stage, frost incidents can cause irreversible damages such as browning or necrosis of flower bud tissues (Rodrigo 2000; Guerriero et al. 2006). During this phase, the vascular connection between the floral organs and bud axis is restored, the xylem vessels reach the ovary and the water moves into the flower tissues. This condition can determine the development of ice crystals that destroy the integrity of the cell walls with the appearance of extra-cellular damages (Proebsting and Andrews 1982; Szalay et al. 1999).

Knowledge of frost tolerance of a given genotype is helpful in order to avoid risks in certain cultivation areas where frost events can occur. To evaluate frost tolerance, artificial freezing procedures have been proposed to reproduce specific climatic conditions which occur in the orchard. In this way, it is possible to detect the endogenous response induced by treatment and to predict possible damage (Palomen and Biszard 1997).

To measure the frost tolerance of tissues, a range of different methods can be used to evaluate the injuries. The visual observation of tissue browning in dissected buds is the most simple and widely-accepted method because it is fast and requires no specialized equipment. Analytical tests, such as triphenyl tetrazolium chloride (TTC), differential thermal analysis (DTA), electrical conductivity, phenolic compound leakage (PCL), nuclear magnetic resonance (NMR) spectroscopy, require expensive equipment and are often used to evaluate injuries after artificial frost stress (Quamme et al. 1982; Chalker-Scott et al. 1989). The PCL test is one of the most suitable experimental procedures to evaluate the cellular membrane integrity of apricot flower buds (Bartolini and Viti 1999; Guerriero et al. 2006).

The inheritance of frost hardiness is a topic of great interest in terms of improving the efficiency of breeding programmes, and selecting new resistant genotypes. Tolerance to freezing temperatures is a complex process depending on genetic and epigenetic attributes. The presence of multiple signal transduction pathways between the initial stress signals and the gene expression explains the complex stress responses observed after the exposure of plants to cold stress (Thomashow 1999). Cold-responsive

genes encoding various signal transduction and regulatory proteins have been identified which might contribute to freezing tolerance by controlling the expression of freezing tolerance genes or by regulating the activity of proteins involved in freezing tolerance (Fursova et al. 2009). Obtaining plants that show increased levels of frost tolerance is generally very difficult, probably because frost tolerance seems to be a complex quantitative trait (Guy 2003). In addition, frost tolerance can vary considerably over a year and among different tissues or parts of the plant (Weiser 1970). Molecular knowledge in response to low temperatures is far from being fully understood in *Prunus* spp., although a possible maternal inheritance on cold stress in sour cherry has been reported (Mathers 2004).

In Mediterranean areas, global climatic changes have led to warm autumn-winter seasons, followed by late spring frosts (Maracchi et al. 2005). Over the last ten years, frequent late frosts have been recorded in the Tyrrhenian coastal area. The most severe event happened in 2003, causing a strong yield reduction, above all in stone fruit species such as peach, plum and apricot (Reggidori 2005). Despite this exceptional event, several apricot selections managed a considerable yield, as a consequence of high flower and/or young fruit survival.

The aim of this research was to test, by a laboratory freezing method, the degree of frost tolerance of several apricot genotypes selected at the University of Pisa (Italy) from different breeding sources. In addition, their parents were studied by exploring their genetic background.

Material and Methods

Plant material and field site

This study considered mature bearing trees from nine apricot selections, obtained by five controlled breeding crosses, and their own parents (Table 1).

These genotypes (four replications), belonging to a wide apricot germplasm collection (cultivars, selections and several F_2 and F_1 progenies), were grown at the experimental station of the Department of Tree Science, Entomology and Plant Pathology (DTSEPP, University of Pisa) located in the Tuscan coastal area (Venturina-Livorno-Italy, altitude 6 m, lat. 43.02° N, long. 10.36° E). Trees grafted on 'Myrobolan 29/C' rootstock were trained as free palmette (5x4 m) with rows facing east-west. The conventional orchard management (pruning, fertilisation, plant protection and drip system irrigation) was carried out in the same way for all genotypes.

Field observations

Trees were monitored recording the flower bud evolution during the autumn-spring seasons in accordance with routine field observations carried out since 1990, within the apricot breeding evaluation program of the DTSEPP (Pisa University). Periodical observations were made to evaluate the evolution of bud phenological stages and, on labelled one-year old fruiting shoots (50 per cultivar), number of flower buds was recorded monthly to calculate the blooming and fruit-set percentage.

Temperature and rainfall were also recorded: daily minimum and maximum hourly temperatures were recorded by the automatic data-logger Tinytag Plus® (Gemini Data Loggers, West Sussex, UK, 2003).

We compared the year 2003, when a severe spring frost occurred, with 2006–2008, typical years without natural spring frosts.

Artificial frost treatment

The Flower Bud (FB) frost tolerance of each genotype was analysed over the three-year period 2006–2008, which were regular years in terms of winter-spring climatic conditions.

FB at deep dormancy (stage A) and swelling phenological stages (stage B–C, pink sepal clearly visible) were considered (Bartolini et al. 2004). FBs were collected from the median-apical portion of one-year-old fruiting shoots randomly cut from the whole canopy.

Before starting the artificial frost treatment, samples of buds were collected from 20–30 shoots in order to evaluate:

- The incidence of flower anomalies. To assess specific symptoms of flower anomalies such as the browning of tissues affecting pistils, stamens or the whole bud, samples of buds (45, arranged as three replications) were bisected longitudinally and observed under a stereo-microscope (Nikon HFX-II) The data, expressed as percentages, represent the total of flower anomalies observed;

Table 1. Apricot selections obtained from five cross breedings using different parents.

Cross breeding	Parents		Selections
	Female	Male	
1	'Canino Tardiva'	'Pisana'	11/15/3
			11/48/7
2	'Farmingdale'	'Amabile Vecchioni'	P1 41/6
			2/29/11
3	'Portici'	'Harcot'	7C 49/5
			7C 33/5
4	'Boccuccia Spinosa'	'Harcot'	11/48/2
			11/19/2
5	'Haggit'	*Prunus brigantiaca* Vill.	7C 20/3

– The relative water content. The fresh and dry weights (one week in the oven at 60 °C) of 25 flower buds (arranged as five replications) were determined to calculate the relative water content (hydration).

The artificial frost treatment was performed on median-apical segments (15–20 cm long) of one-year-old fruiting shoots, which were kept in a freezing chamber and exposed at a temperature of −4 °C (for 2 h) achieved by a continuous chilling decrease (2 °C h^{-1}). This temperature was chosen because it is the most recurrent critical temperature occurring during spring time in the Tuscan coastal area where the experimental fields were located (ARSIA: http://agrometeo.arsia.toscana.it/index.php).

The frost sensitivity was determined by the Phenolic Compound Leakage (PCL) test, which is able to estimate the level of integrity of the cellular membranes of tissues (Chalker-Scott et al. 1989). The 2-hour subzero treatment (Viti et al. 1994) was followed by a gradual thaw by incubation at room temperature for 4 h under light (300–400 μE m^{-2} s^{-1}). One group of one-year-old fruiting shoots was kept in natural conditions as a control. Next, 30 flower buds (three replications) were collected and placed in distilled H_2O and incubated for 24 h in a shaker at 23 °C under continuous light (300–400 μE m^{-2} s^{-1}). At the end of this treatment, the first PCL determination was carried out measuring the absorbance at 260 nm using a UV-Vis spectrophotometer; samples were then kept at −20 °C for 12 h to destroy the integrity of the cellular membranes. After thawing, the same samples were shaken for 24 h, and PCL was measured again (second determination). PCL was expressed as a percentage of the second value (Chalker-Scott et al. 1989; Bartolini and Viti 1999).

In relation to the PCL percentages, the genotypes were grouped as: i) very frost-tolerant ≤ 20 %; ii) frost-tolerant 20–50 %, and iii) frost-sensitive > 50 %. The range of tolerance level was scored considering that the PCL measures the loss of the cellular membrane integrity and indirectly the degree of tissue damage. The PCL rate has been related to the survival of bud and consequently to the bearing aptitude. In apricot, it has been defined that bud injury percentages below 20 % are not influent on the current yield while percentages above 50 % are able to determine a strong yield reduction (Viti and Monteleone 1993).

Statistics

Percentages were converted into angle values by Arcsin transformation and the analysis of variance (ANOVA) was performed to show the relation between the genotypes and the fresh weight, hydration and PCL of flower buds. Differences between means were considered statistically significant at $P \leq 0.05$ according to Newman-Keuls. Student's t-test was used to compare data from frost-treated and untreated flower buds ($P \leq 0.05$). A correlation analysis of PCL versus fruit set was carried out and correlation coefficients were reported in terms of goodness of fit. The analyses were performed using STATGRAPHICS Plus 5.1 software (Manugistics, Inc.).

Results

Field observations

In Fig. 1 the fortnightly mean minimum temperatures recorded in 2003 and 2006–08 from January to April are compared. The 2003 year was characterised by extraordinary frost events, reaching absolute minimum temperatures of −4.9 °C in late February and −4.5 °C in early April.

These late frost temperatures, happened during the blooming-fruit set time, have caused heavy damages, limiting the yield in DTSEPP apricot germplasm collection. However, several selections had a considerable yield as a consequence of the high flower and/or young fruit survival. After the frosts of 2003, among the selections tested, the 11/15/3, 11/48/7, 7C 49/5, 11/19/2, 11/48/2 and 7C 20/3 showed a fruit set entity higher or similar than the mean values recorded during the three-year period 2006–08 (Fig. 2). In particular, selections 7C 49/5, 11/19/2, 11/48/2 and 7C 20/3 had a fruit set entity significantly higher (≥ 60 %). The better performance of these selections was also confirmed by the low fruit set entity (< 20 %) of the remaining genotypes (selections and parents) recorded in 2003.

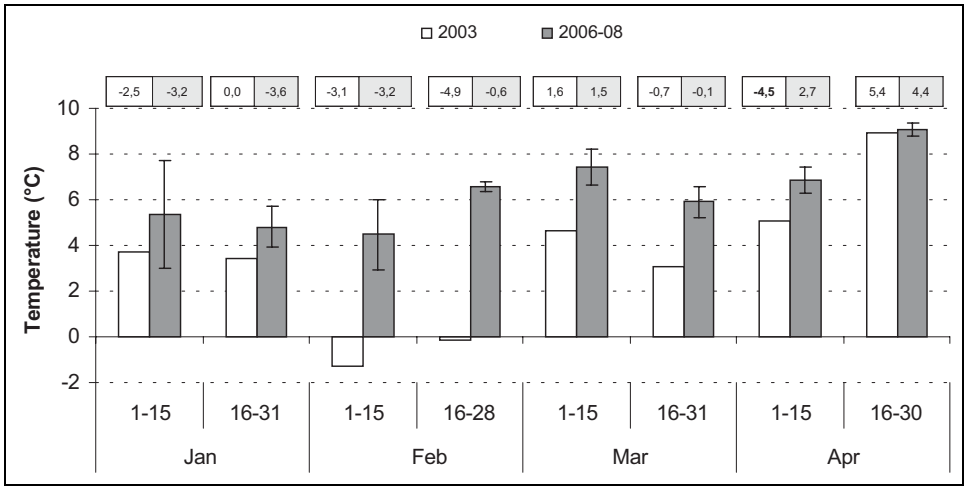


Fig. 1. Mean of minimum temperatures recorded from January to April at 15-day intervals: 2003 (white column) and 2006–2008 (grey column, ± standard error of mean). At the top the absolute minimum temperatures reached during the 15-day intervals are showed.

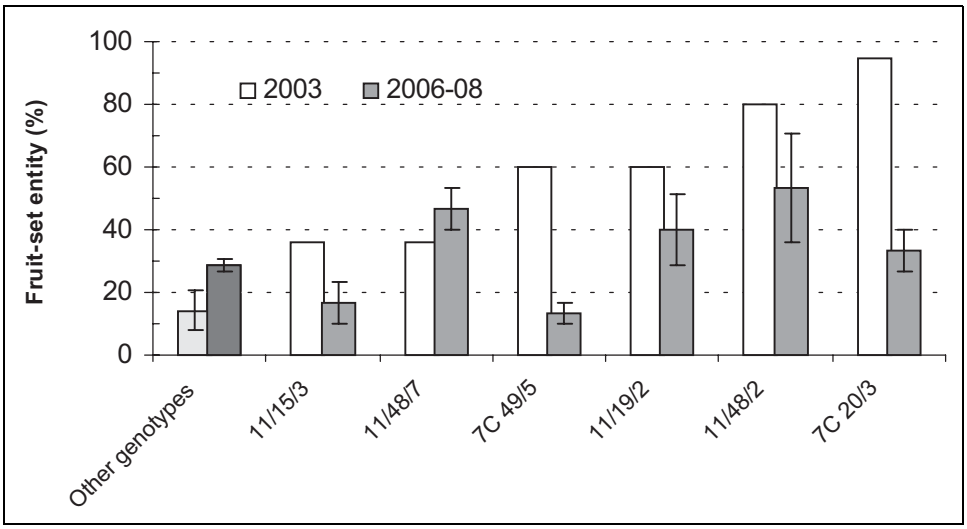


Fig. 2. Fruit set percentage: 2003 (white column) and mean of 2006–2008 (grey column). The best apricot selections are compared with the mean data of the remaining genotypes (selections and parents). Values are means ± standard error of mean.

Appearance of floral anomalies under field conditions

The floral anomalies, recorded during 2006–2008 on flower buds from natural field conditions, showed different percentage values of damaged buds with respect to genotype and phenological stages (Fig. 3).

However, the anomalous buds did not exceed the threshold of 20 % at the two phenological stages. Several genotypes (2/29/11, 'Portici', 'Haggit', 11/37/3, P1 41/6 and 'Harcot') were characterized by a very low rate of anomalies (< 10 %). Another group of genotypes was detected, which showed the highest rates (13–20 %) of anomalies only at the dormant bud stage (7C 33/5, 'Amabile Vecchioni', 'Farmingdale', 11/19/2). These genotypes had a percentage of anomalies closer to zero when the flower buds were at the phenological stage of swelling.

Frost tolerance evaluation

For 2006–2008, the PCL recorded on untreated dormant flower buds (stage A), ranged from 10 % to 30 % (Fig. 4). Most genotypes had rates below 20 %, while 11/19/2, 'Pisana', P1 41/6 and 'Farmingdale' went over this percentage with statistically significant differences compared to the remaining genotypes. The frost treatment (−4°C) did not cause a significant PCL increase in comparison with the untreated buds, with the exception of P1 41/6.

At stage B–C, 2/3 of genotypes showed a marked increase in PCL values and exceeded the 20 % threshold (Fig. 5).

After frost treatment, the swelling FB showed a leakage percentage ranging from 11 % to 83 %. The greatest frost hardiness was found in 'Pisana', 7C 20/3, 11/48/7, *P. brigantiaca* Vill. and 11/19/2, which after artificial sub-zero treatments, did not show significant injuries compared to the control buds. The frost-tolerant group was represented by 'Amabile Vecchioni', 7C 49/5, 11/15/3, 11/48/2, 7C 33/5, 'Harcot', 'Portici', 2/29/11 and 'Haggit'. The frost-sensitive group included 'Boccuccia Spinosa', P1 41/6, 'Farmingdale', and 'Canino Tardiva'. Within this group, P1 41/6, 'Farmingdale' and 'Canino Tardiva' were the most susceptible as denoted by the highest PCL percentage (> 60 %), meaning a strong loss of cell integrity.

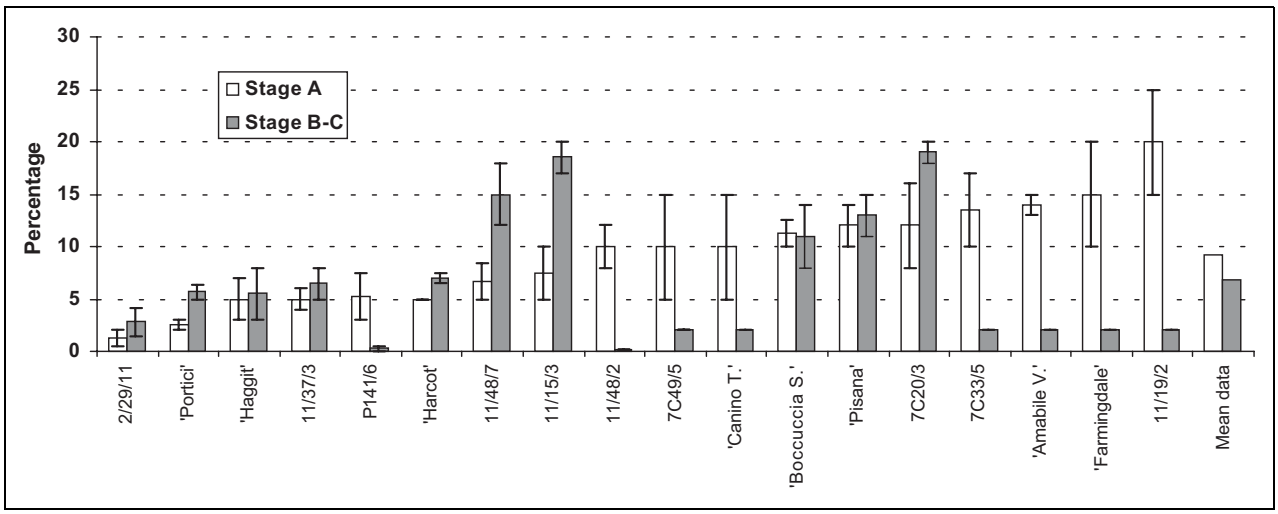


Fig. 3. Apricot selections and parents: percentage flower bud anomalies recorded in natural field conditions at phenological stage A (white column) and B–C (grey column). Values are means (± standard error of mean) of 2006–2008.

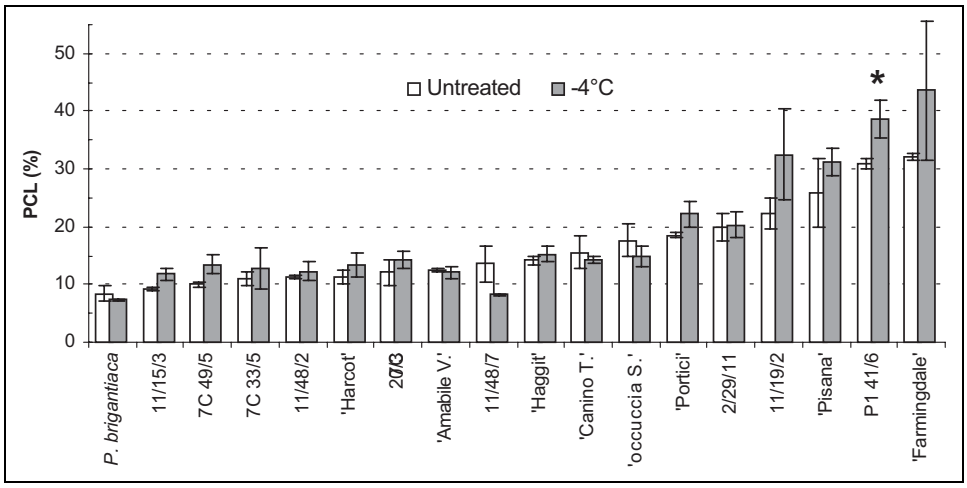


Fig. 4. Apricot selections and parents: phenolic compound leakage (PCL) percentage of untreated (white column) and frost treated (−4 °C, grey column) apricot flower buds at phenological stage A. Values are means (±standard error of mean) of 2006–2008. The asterisk denotes significant differences between untreated and frost-treated flower buds (Student t test, $P \leq 0.05$).

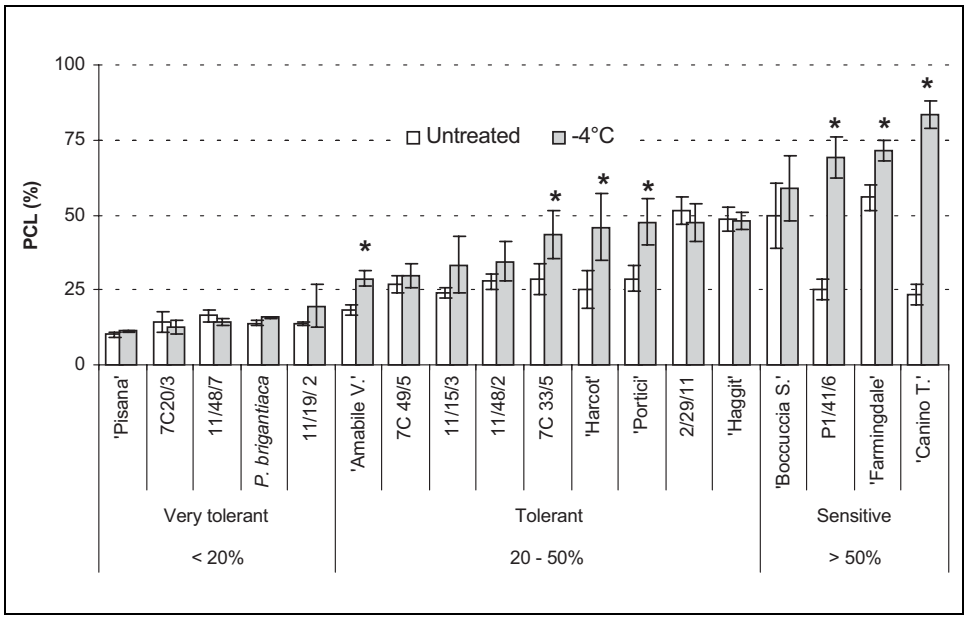


Fig. 5. Apricot selections and parents: phenolic compound leakage (PCL) percentage of untreated (white column) and frost treated (−4 °C, grey column) apricot flower buds at phenological stage B–C. Genotypes are grouped in relation to degree of frost hardiness of flower buds: i) very frost-tolerant (≤ 20 %); ii) frost-tolerant (20–50 %); iii) frost-sensitive (> 50 %). Values are means (± standard error of mean) of a three-year period (2006–2008). The asterisk denotes significant differences between untreated and frost treated flower buds (Student t test, $P \leq 0.05$).

The differences in the degree of frost sensitivity were seemingly unrelated to the size of the flower buds at the phenological stage A, the most suitable for representing the genotypic trait (Table 2). In fact, between the sensitive (S) and the very tolerant (VT) genotypes, no significant differences in flower bud fresh weight were found. The tolerant genotypes (T) showed a large variability, with a mean flower bud fresh weight that varied between 8,7 and 14,4 mg for 7C 33/5 and 'Amabile Vecchioni', respectively. This relevant variability, between and within the cross-breeding groups, was also observed by analyzing the flower bud hydration percentage during the transition from deep (stage A) to the end of dormancy (stage B).

However, considering the general average of genotypes grouped according to the degree of frost sensitivity, the very tolerant (VT) genotypes showed the lowest mean hydration increase.

With regard to the breeding groups, when the parents had a strong difference in frost tolerance (i.e: S × VT), the progenies were generally characterized by a lower degree of frost sensitivity which was similar to the trait of the male parent.

Correlation between 2003 fruit-set entity and PCL

Analyzing the fruit-set entity recorded after the spring frost of 2003 and the flower bud frost-tolerance degree of selections (expressed as PCL percentages artificially tested at stage BC) a satisfactory correlation ($R^2 = 0{,}685$) was observed (Fig. 6). Two genotypes, P1 41/6 and 7C 20/3, were characterized by opposite responses. P1 41/6 was the most susceptible to the spring frost, showing a very low fruit-set entity (4 %) associated with a very high percentage of PCL (69 %); 7C 20/3 was the most tolerant genotype, characterised by the highest fruit-set entity (95 %) and very low bud tissue injuries (13 % PCL).

Discussion

The results revealed that the exposure of apricot flower buds to an artificial freezing temperature of −4°C can induce a different degree of frost tolerance in relation to the phenological stage. This temperature is the most recur-

Table 2. Apricot genotypes of the five breading groups. Degree of frost sensitivity according to PCL test applied to flower buds at phenological stage B–C: Sensitive = S, Tolerant = T, Very Tolerant = VT; flower bud fresh weight (mg) recorded at phenological stage A; hydration percentage expressed by the increase from stage A to stage B–C, before artificial frost treatment.

Cross breading	Genotypes	Frost sensitivity	Fresh weight (mg)	Hydration increase (%)
Group 1	11/48/ 7	VT	12.3 a	10.6 bc
'Canino T.' × 'Pisana'	'Canino T.'	S	8.4 b	21.3 ab
	11/15/3	T	11.3 a	6.1 c
	'Pisana'	VT	8.3 b	27.3 a
Group 2	2/29/11	T	9.4 b	36.3 a
'Farmingdale' × 'Amabile V.'	'Amabile V.'	T	14.4 a	28.0 a
	P1 41/6	S	15.0 a	30.4 a
	'Farmingdale'	S	13.4 ab	17.7 b
Group 3	7C33/5	T	8.7 n.s.	26.3 n.s.
'Portici' × 'Harcot'	'Harcot'	T	9.3	30.1
	7C49/5	T	8.9	28.8
	'Portici'	T	12.8	20.9
Group 4	11/48/2	T	10.2 n.s.	26.9 n.s.
'Boccuccia S.' × 'Harcot'	'Harcot'	T	9.3	30.1
	11/19/2	VT	14.3	28.9
	'Boccuccia S.'	S	13.0	23.1
Group 5	7C20/3	VT	8.0 c	22.5 b
'Haggit' × *P. brigantiaca*	'Haggit'	T	11.0 b	30.2 b
	P. brigantiaca	VT	19.0 a	10.1 a
General mean		S	12.4 ± 2.8	23.1 ± 5.3
		T	10.5 ± 2.9	26.4 ± 8.1
		VT	12.4 ± 4.6	19.9 ± 9.0

'Canino Tardiva': 'Canino T.'; 'Amabile Vecchioni': 'Amabile V.'; 'Boccuccia Spinosa': 'Boccuccia S.'; *Prunus brigantiaca: P. brigantiaca*; Values followed by different letters in a column for each cross breeding group are significantly different at $P \leq 0.05$.; n.s.: not significant. General mean values of each frost sensitivity group are followed by standard deviation (±).

rent minimal value recorded over the last ten-year period under the environmental conditions of the Tuscan coastal area (ARSIA source). When late frosts occurred, temperatures close to −4°C destroyed the flower bud, causing considerable damage to floral tissues with the consequent failure in yield (Guerriero et al. 2006).

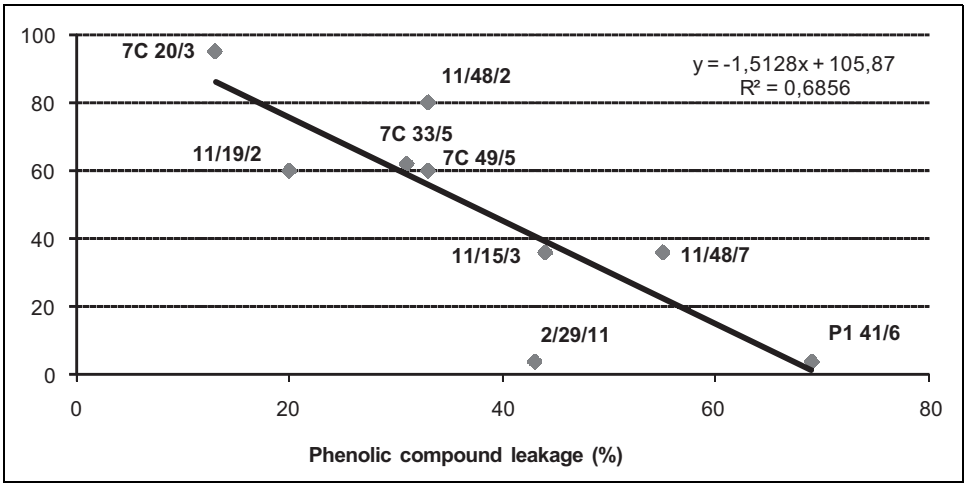


Fig. 6. Correlation between fruit set percentage detected in several apricot seedlings after spring frost of 2003 and percentage phenolic compound leakage (mean values of the three-year period 2006–2008) recorded on flower buds at phenological stage B–C after frost treatment (−4 °C) ($y = -1.5128x + 105.87$; $R^2 = 0.6856$).

The artificial frost temperature applied to dormant flower buds did not cause significant injuries, as indicated by the low PCL values, which were similar to those recorded for untreated buds. The dormant stage tolerance was confirmed by the visual observations of floral organs carried out on buds collected from the orchard. During autumn-winter, all the genotypes were characterized by a low floral anomaly rate (below 20 %), which we believe is of physiological origin rather than due to climatic factors. This is because during 2006–2008 temperatures were never low enough to increase the damage in floral bud tissues. The genotypes characterized by floral anomaly percentages close to 20 %, also presented a very low rate of anomalous buds at the pre-flowering stage. These results were in accordance with previous studies (Viti and Monteleone 1993, 1995) showing that, throughout the autumn-winter season, the anomalous buds can have several drop waves which then lead to a physiological selection of the flower buds. In consequence, only the best ones manage to bloom. However, at the B–C stages, in most genotypes the artificial frost test significantly increased the PCL percentage, compared to the dormant stage. When flower buds reach the pre-flowering stages, they become particularly sensitive to spring frosts (Rodrigo 2000; Szalay et al. 2006). This is a consequence of the morphological and physiological status of swelling buds, characterized by a fast dehardening against sub-zero temperatures (Ashworth and Rowse 1982). In this physiological status, heat temperatures supported the reactivation of bud growth, in concomitance with the vascular continuity established between the floral primordium and the fruiting shoot (Bartolini et al. 2006b). Thus, we have confirmed that apricot is a sensitive species to freezing temperatures occurring when flower buds have fulfilled the endodormancy with the beginning of active growth (Rodrigo 2000; Bartolini et al. 2006a).

In accordance with previous results (Bartolini and Viti 1999; Guerriero et al. 2006), a different PCL value was observed for each genotype studied, allowing to classify them into three groups: i) very frost-tolerant; ii) frost-tolerant and iii) frost-sensitive. Within the very frost-tolerant group, 7C 20/3, 11/48/7 and 11/19/2 were the most remarkable genotypes, thus confirming the good yield recorded after the severe spring frost of 2003. This result was corroborated by a satisfactory correlation between fruit-set entity and PCL values.

At the end of winter, xylem vessel differentiation is completed with an increase in water content and a general rise in tissue hydration of the swelling flower buds. A loss of cellular membrane integrity occurs following ice formation within the intracellular spaces (Ashworth 1984). However, from our experimental trials, a clear relationship between the increase in hydration and floral tissue injury rate was not always observed. It is likely that apricot genotypes can react differently against frost stress in different ways. Examples of this, include the ability to deep supercool, maintaining water and dilute solution as metastable liquids below their melting points (Ashworth 1984), or activating scavenging mechanisms enhancing the cells' resistance to frost, such as the formation of antioxidant enzymes (e.g. SOD) and an increase in GSH/GSSG ratio (Doulis et al. 1993; Bartolini et al. 2006a).

Considering the genetic feature relating to the inheritance of the frost tolerance traits, this study suggests a possible implication of the male parent as observed for 7C 20/3, 11/48/7 and 11/19/2 which have *P. brigantiaca* Vill., 'Pisana' and 'Harcot' respectively as the father. *P. brigantiaca* Vill. is a genotype that is well known for its frost tolerance, mainly due to a very late flowering (Gorina and Richter 2000).

However, these results did not permit to establish a clear mechanism concerning frost-tolerance inheritance, due to the complexity of the issue, and the influence of environmental factors (Guy 2003). Further studies in order to understand how apricot inherits frost tolerance are required, which should, among others, involve a greater number of seedlings.

The artificial frost stress method, which eliminates the multiple variables of natural conditions, turned out to be suitable for determining apricot's tolerance to winter and early spring frosts. The PCL test, by evaluating cell integrity, enabled us to determine the frost tolerance of flower buds and the degree of bud injuries. The artificial frost treatment associated with the PCL test ascertained the performance of genotypes against frost tolerance in relation to an established threshold of temperature. Consequently, this methodology could be used to accelerate breeding programs aiming at the development of new cultivars of frost-hardy apricot.

Acknowledgements

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Addresses of authors: Raffaella Viti (corresponding author), Dipartimento di Difesa e Coltivazione delle Specie Legnose "G. Scaramuzzi", Università di Pisa, Via del Borghetto 80, 56124 Pisa, Italy, and Susanna Bartolini and Lucia Andreini, Scuola Superiore 'S. Anna' di Studi Universitari e di Perfezionamento, Piazza Martiri della Libertà, 33, 56126 Pisa, Italy, e-mail: rviti@agr.unipi.it.