Resistance to Plum Pox Virus (Dideron Isolate RB3.30) in a Group of California Almonds and Transfer of Resistance to Peach

P. Martínez-Gómez, M. Rubio, and F. Dicenta
Departamento de Mejora y Patología Vegetal, Centro de Edafología y Biología Aplicada del Segura-Consejo Superior de Investiguaciones Científicas, P.O. Box 4195, E-30080 Murcia, Spain

T.M. Gradziel
Department of Pomology, University of California, One Shields Avenue, Davis, CA 95616

ABSTRACT. Sharka [(plum pox virus (PPV))] mainly affects Prunus species, including apricot (Prunus armeniaca L.), peach (Prunus persica L.), plum (Prunus salicina Lindl., Prunus domestica L.), and, to a lesser degree, sweet (Prunus avium L.) and sour cherry (Prunus cerasus L.). Level of resistance to a Dideron isolate of PPV in seven California almond [P. dulcis (Miller) D.A. Webb], five processing peach cultivars, and two peach rootstocks was evaluated. In addition, almond and peach selections resulting from interspecific almond × peach hybridization and subsequent gene introgression were tested. Evaluations were conducted in controlled facilities after grafting the test genotypes onto inoculated GF305 peach rootstocks. Leaves were evaluated for PPV symptoms during three consecutive cycles of growth. ELISA-DASI and RT-PCR analysis were also employed to verify the presence or absence of PPV. Peach cultivars and rootstocks showed sharka symptoms and were ELISA-DASI or RT-PCR positive for some growth cycles, indicating their susceptibility to PPV. Almond cultivars and almond × peach hybrids did not show symptoms and were ELISA-DASI and RT-PCR negative, demonstrating resistance to PPV. Two (almond × peach) F1 selections as well as two of three backcrossed peach selections also showed a resistant behavior against the PPV-D isolate. Results demonstrate a high level of resistance in almond and indicate potential for PPV resistance transfer to commercial peach cultivars.

Materials and Methods

PLANT MATERIALS. Plant material evaluated included seven almond cultivars, five processing peach cultivars, and two peach rootstocks (Table 1). Also tested were genotypes resulting from interspecific almond × peach hybridization as well as subsequent backcrossing and selfing with selection for peach fruit types.

PPV ISOLATE. PPV isolate RB3.30 was used as virus inoculum and is a Dideron Type isolate obtained in Spain from the plum ‘Red Beaut’. The isolate is maintained at the Instituto Valenciano de Investigaciones Agrarias (IVIA) Valencia, Spain (Asensio, 1996).
EVALUATION PROCEDURE. Evaluation experiments were carried out in a sealed greenhouse in Murcia (Spain), following procedure described by Martínez-Gómez and Dicenta (1999). Scions were propagated onto infected symptomatic GF305 peach seedlings (one scion per seedling). GF305 peach is characterized by its susceptibility to PPV (Bernhard et al., 1969). Following 4 months of growth, scion-grafted trees were forced into dormancy by subjecting them to 7 °C and darkness for 2 months. After this cold dark treatment, trees were moved to an insect-proof greenhouse for 4 months. Three cycles of evaluation were performed over 2 years. The number of plants evaluated depended on scion graft success as only plants where the GF305 rootstock showed unambiguous PPV symptoms were considered as successfully inoculated. During each growth cycle leaf symptoms were scored from 0 (no symptoms) to 5 (maximum intensity of symptoms as observed on GF305 rootstock) at 2 months following budbreak. PPV symptoms evaluated include chlorotic discoloration of expanding and mature leaves and deformations of leaf tips and margins (Fig. 1). ELISA-DASI or RT-PCR positive reactions and the presence of disease symptoms in leaves in any cycle indicated the susceptibility of the genotype.

ELISA-DASI ANALYSIS. To ascertain the presence or absence of PPV in samples, an ELISA-DASI (Double Antibody Sandwich Indirect) assay was applied to the leaves during the first and third growth cycles using the 5B monoclonal antibody against the coat protein of PPV (Cambra et al., 1994). Optical densities (OD) at 405 nm were recorded after 60 min. In accordance with Sutula et al. (1994), OD readings were considered positive when above the 0.6 arbitrary units cut-off value. 

Table 1. Plant material evaluated including the origin, use, and main horticultural characteristics; BC = backcrossed.

| Genotype     | Origin                  | Use         | Endocarp | Mesocarp color | Mesocarp texture | Flowering time |
|--------------|-------------------------|-------------|----------|----------------|------------------|---------------|
| Carmel       | Nonpareil × Mission     | Almond      | Paper    | White          | Almond-like      | Very early    |
| Mission      | Early California selection | Almond    | Hard     | White          | Almond-like      | Early         |
| Ne Plus Ultra| Early California selection | Almond    | Soft     | White          | Almond-like      | Very early    |
| Nonpareil    | Early California selection | Almond    | Paper    | White          | Almond-like      | Early         |
| Padre        | Mission × Swanson       | Almond      | Paper    | White          | Almond-like      | Early         |
| Price        | Nonpareil × Mission     | Almond      | Paper    | White          | Almond-like      | Early         |
| Sonora       | Nonpareil × Eureka (BC) | Almond      | Paper    | White          | Almond-like      | Very early    |
| Andross      | Early California selection | Processing peach | Hard     | Yellow        | Peach-like      | Late          |
| Bolinha      | Brazilian selection     | Processing peach | Very hard | Yellow      | Peach-like      | Early         |
| Dr Davis     | California selection    | Processing peach | Hard     | Yellow        | Peach-like      | Late          |
| Halford      | Lovell seedling         | Processing peach | Very hard | Yellow       | Peach-like      | Late          |
| Ross         | California selection    | Processing peach | Very hard | White        | Peach-like      | Late          |
| Lovell       | Early California selection | Peach rootstock | Very hard | White        | Peach-like      | Late          |
| Nemaguard    | P. davidiana × peach    | Peach rootstock | Very hard | White        | Intermediate    | Early         |
| Hansen 536   | Almond × peach          | Hybrid rootstock | Very hard | White        | Intermediate    | Early         |
| Nickels      | Almond × Nemaguard      | Hybrid rootstock | Very hard | White        | Intermediate    | Early         |
| 54P455       | Peach selection         | Peach breeding line | Hard     | Yellow       | Peach-like      | Early         |
| 7926-1       | Padre almond × 54P455   | Hybrid breeding line | Very hard | White        | Intermediate    | Early         |
| F10C,20-51   | (Padre × 54P455) _F_2_  | Almond breeding line | Paper    | White        | Peach-like      | Early         |
| F10C,12-28   | (Padre × 54P455) _F_2_  | Almond breeding line | Paper    | White        | Peach-like      | Early         |
| F8,5-156     | (Peach × F10C,12-28) _F_ | Peach breeding line | Very hard | Yellow | Peach-like      | Early         |
| F8,5-166     | (Peach × F10C,12-28) _F_ | Peach breeding line | Very hard | Yellow | Peach-like      | Early         |
| 99,15-154    | (Peach × Nonpareil) _BC_ | Peach breeding line | Very hard | Yellow | Peach-like      | Late          |

Fig. 1. Disease response following grafting onto PPV infected rootstock. (A) Absence of PPV symptoms in Nonpareil almond grafted onto GF305 showing strong symptoms of the disease (indicated by arrows). (B) PPV symptoms (indicated by arrows) in the Nemaguard peach grafted onto GF305 also showing symptoms of the disease.
al. (1986), samples with OD at least double those of the healthy control were considered ELISA-positive.

**RT-PCR Analysis.** RT-PCR analysis (Wetzel et al., 1991) was carried out using total RNA extracted using the RNeasy Plant Mini Kit (Qiagen, Valencia, Calif.) as described by MacKenzie et al. (1997). Two specific primers within the coat protein (CP) gene, VP337 (CTCTGTGTCCTCTTCTTTG) complementary to 9487-9508 positions of genomic PPV and VP338 (CAATAAAGCCATTGTTGATC) homologous to 9194 to 9216 positions, were assayed. PCR parameters were: one cycle at 94 °C for 2 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, and finally with an extension temperature at 72 °C for 5 min (Martínez-Gómez et al., 2003a). Amplified products were electrophoresed in 1% agarose gels in 40 mM Tris-acetate and 1 mM EDTA, pH 8.0 (TAE) and stained with ethidium bromide. A 1-kb plus DNA Ladder (Invitrogen Life Technologies) was used as molecular size standard.

**Results**

All almond cultivars grafted onto previously inoculated GF305 peach rootstocks showed resistance to the PPV-D isolate assayed after three cycles of study (Table 2). They did not show any symptoms and were ELISA-DASI and RT-PCR negative (Fig. 2) despite the symptoms observed in the GF305 rootstock.

Processing peach cultivars Andross, Bolinha, Dr. Davis, Halford, and Ross, peach breeding parent '54P455', and the peach rootstocks Lovell and Nemaguard, were susceptible to the PPV-D isolate assayed. Symptomatic plants developed chlorotic discoloration and distortion of leaves characteristic of PPV (Fig. 1) and assayed positive by ELISA-DASI or RT-PCR during at least one of the three growth cycles assayed (Table 2).

Interspecific almond x peach hybrids, including the 'Hansen 536' and 'Nickels' rootstocks, and the ('Padre' x '54P455' peach) hybrid '7926-1', demonstrated resistance. Six of the eight almond x peach derived genotypes also showed a resistant response to the PPV-D isolate assayed. Peach-type selection '99,15-154' and 'F8,5166' developed PPV symptoms.

Symptomatic plants were always associated with high ELISA-DASI OD values (Table 2). Four of the symptomatic and ELISA-DASI positive genotypes also gave positive RT-PCR responses while three other symptomatic ELISA-DASI positive genotypes gave negative RT-PCR responses.

**Table 2.** Evaluation of resistance of genotype assayed to plum pox virus (PPV)-D isolate RB3.30 of PPV.

| Genotype     | Cycle 1 | Cycle 2 | Cycle 3 |
|--------------|---------|---------|---------|
|              | Mean intensity | ELISA-DASI | Mean intensity | ELISA-DASI | Mean intensity | ELISA-DASI |
| Plants Symptomatic of symptoms | Symptomatic plants | Positive plants | OD$_{405}$ | Plants Symptomatic of symptoms | Symptomatic plants | Positive plants | OD$_{405}$ | Plants Symptomatic of symptoms | Symptomatic plants | Positive plants | OD$_{405}$ | RT-PCR |
| Almond       |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Carmel       | 3       | 0       | 0       | 0.06    | 0       | 0       | 0       | 0.06    | –        |         |         |         |         |
| Mission      | 4       | 0       | 0       | 0.10    | 0       | 0       | 0       | 0.06    | –        |         |         |         |         |
| NePlusUltra  | 1       | 0       | 0       | 0.10    | 0       | 0       | 0       | 0.06    | –        |         |         |         |         |
| Nonpareil    | 3       | 0       | 0       | 0.10    | 0       | 0       | 0       | 0.06    | –        |         |         |         |         |
| Padre        | 2       | 0       | 0       | 0.10    | 0       | 0       | 0       | 0.06    | –        |         |         |         |         |
| Price        | 1       | 0       | 0       | 0.08    | 0       | 0       | 0       | 0.05    | –        |         |         |         |         |
| Sonora       | 1       | 0       | 0       | 0.10    | 0       | 0       | 0       | 0.06    | –        |         |         |         |         |
| Peach        |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Andross      | 4       | 0       | 0       | 0.11    | 0       | 0       | 1       | 1.0     | 0.35     | +        |         |         |         |
| Bolinha      | 3       | 1       | 1.0     | 1.40    | 0       | 0       | 0       | 0.06    | –        |         |         |         |         |
| Dr. Davis    | 5       | 2       | 1.0     | 2.63    | 0       | 0       | 1       | 1.0     | 0.20     | –        |         |         |         |
| Halford      | 3       | 1       | 1.0     | 0.59    | 0       | 0       | 1       | 1.0     | 0.18     | +        |         |         |         |
| Ross         | 4       | 0       | 0       | 0.09    | 0       | 0       | 1       | 1.0     | 0.20     | –        |         |         |         |
| Peach rootstock |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Lovell       | 3       | 1       | 2.0     | 1.14    | 0       | 0       | 0       | 0.06    | –        |         |         |         |         |
| Nemaguard    | 2       | 2       | 2.0     | 1.89    | 0       | 0       | 1       | 1.0     | 0.20     | –        |         |         |         |
| Hybrid rootstock |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Hansen 536   | 3       | 0       | 0       | 0.06    | 0       | 0       | 0       | 0.06    | –        |         |         |         |         |
| Nickels      | 3       | 0       | 0       | 0.06    | 0       | 0       | 0       | 0.06    | –        |         |         |         |         |
| Breeding lines |         |         |         |         |         |         |         |         |         |         |         |         |         |
| 54P455       | 5       | 3       | 1.5     | 3.71    | 0       | 0       | 3       | 1.5     | 0.46     | +        |         |         |         |
| 7926-1       | 1       | 0       | 0       | 0.07    | 0       | 0       | 0       | 0.07    | –        |         |         |         |         |
| F10C,20-51   | 3       | 0       | 0       | 0.08    | 0       | 0       | 0       | 0.06    | –        |         |         |         |         |
| F10C,12-28   | 4       | 0       | 0       | 0.08    | 0       | 0       | 0       | 0.06    | –        |         |         |         |         |
| F8,5-156     | 2       | 0       | 0       | 0.06    | 0       | 0       | 0       | 0.05    | –        |         |         |         |         |
| F8,5-166     | 5       | 0       | 0       | 0.10    | 1       | 2.0     | 0       | 0       | 0.27     | +        |         |         |         |
| 99,15-134    | 3       | 1       | 2.0     | 1.68    | 0       | 0       | 1       | 1.0     | 0.27     | +        |         |         |         |

*Positive (+) or negative (–) reaction.

*OD$_{405}$ = optical density at 405 nm values after 60 min. Mean OD$_{405}$ in infected and healthy GF305 peach rootstocks were 1.80 and 0.07, respectively.

*Intensity: 0 = no symptoms to 5 = maximum intensity of leaf chlorosis and distortion. Mean intensity of PPV symptoms in infected GF305 peach rootstocks was 3.0.
Mean intensity of PPV symptoms of all the infected peach genotypes was 1.4. This intensity is very low in comparison to the intensity of symptoms as observed in the replications of each cultivar during the third cycle of the study. All plants showing symptoms also gave positive ELISA-DASI readings. In addition, detection of PPV by RT-PCR during the third cycle confirmed the results obtained by ELISA-DASI. All samples that were positive by RT-PCR were also positive by ELISA-DASI. However, in the cases of lower OD of ELISA-DASI (peach cultivars Dr. Davis and Ross, and Nemaguard rootstock), the RT-PCR was negative. While a higher sensitivity has been reported for the RT-PCR in comparison to the ELISA-DASI (Candresse et al., 1994; Martínez-Gómez et al., 2003a; Wetzel et al., 1991), the erratic distribution of PPV common in infected Prunus tissue together with the presence of PCR inhibitors described by Olmos et al. (2002) in some Prunus tissues could have contributed to false RT-PCR negatives.

The level of resistance to the PPV-D isolate of all the California almond cultivars assayed support Dicента et al. (2003), Pascal et al. (2003), and Rubio et al. (2003), who reported the resistance of selected European almond cultivars to PPV-D and PPV-M isolates. Dallot et al. (1997), detected the PPV virus by ELISA in ‘Aï’ almond cultivar, after graft-inoculation with five Dideron, three Marcus, and one El Amar isolates. However, the ODs they obtained were low, particularly in the almond inoculated with PPV-D isolates. Only one isolate induced some chlorotic discoloration of the leaves of ‘Aï’, which rapidly disappeared. Dallot et al. (1997) also demonstrated a lower rate of infection by PPV-D isolates, as described previously by Quiot et al. (1995) in apricot and peach. While ‘Aï’, may represent a particular case of susceptibility among almond cultivars, Dallot et al. (1997) did not find any ELISA-positive samples after analyzing 356 trees in a field survey.

Results support the low potential of the almond genotypes used in this study as virus sources in sharked epidemics where Type D isolates of PPV are involved. The almond cultivars used in this study represent ≈70% of current production in California, with most remaining commercial varieties being the progeny of crosses between the resistant ‘Nonpareil’ and ‘Mission’ varieties (Martínez-Gómez et al., 2003b). Type D, which is the most readily transmitted isolate, is the major isolate found in Western Europe and the only isolate reported in North and South America (Dammelsteedt et al., 2001). In both Western Europe and North America, the control of PPV is through widespread and recurrent visual and ELISA-DASI based surveys of existing orchards with tree removal and quarantine restrictions when PPV is found. Confirmation of a freedom from PPV in remaining almond cultivars planted in California could lead to the exclusion of these almond varieties as a potential virus reservoir. In California, where plantings of these almond varieties account for ≈180,000 ha, their removal as a potential host species would allow a more efficient focusing of virus surveys to susceptible Prunus crops.

These findings support the hypothesis that transfer of some level of PPV resistance from almond to peach breeding lines is possible. All almond × peach hybrids as well as six of the eight genotypes derived from interspecific hybridizations were resistant to PPV. The absence of any formidable crossing barriers in either
the initial hybridization or subsequent backcrosses between peach and almond (Gradziel et al., 2001; Gradziel, 2003) further supports the suitability of almond germplasm for peach improvement. Two resistant breeding lines, ‘F10C.20-51’ and ‘F10C.12-28’, have an almond-type tree and nut, and were selected for their high level of self-compatibility derived from the peach parent. The resistant selection ‘F8.5-156’ and the susceptible selection ‘F8.5-166’ have a peach-type tree and fruit and were selected for good canning quality and uniform fruit ripening within the tree. All selections resulted from interspecific hybridization between the resistant ‘Padre’ almond and the susceptible ‘54P455’ peach. The quarantine safeguards required for PPV testing limited the number of peach and almond selections for this initial evaluation. The selections ‘54P455’ peach, ‘7926-1’ interspecific hybrid, and derived progeny were selected for testing since this was the population used for developing the genetic map for peach and almond (Bliss et al., 2002; Foolad et al., 1995) and our eventual goal is to map the resistance gene(s) in almond. The lack of native sources of resistance within peach (Dobša et al., 1994; Escalettes et al., 1998; Gabova, 1994; Pascal et al., 2003) also make almond species a valuable source of PPV resistance for peach species, as previously proposed by Gradziel (2003) and Pascal et al. (2003). Pascal et al. (2003) have reported resistance to PPV Type M in several *P. davidiana* lines, but Moing et al. (2003) had indicated that poor fruit quality is transmitted from *P. davidiana* to peach, which was not a problem in advanced almond-derived peach selections (Gradziel, 2003).

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