Walnut cultivar performance of cold resistance in south central France

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Walnut Cultivar Performance of Cold Resistance in South Central France

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Abstract

In south-central France, walnut exhibited freezing tolerance by acclimation in the fall and deacclimation in the spring. The cold hardiness level varied with the different cultivars and dates. Cold tolerance performances of 7 walnut fruit cultivars (Chandler, Franquette, Fernor, Fernette, Lara, Pedro, Serr) and of the hybrid *Juglans regia* x *Juglans nigra* NG38 were compared in the same location, at the INRA Center of Clermont-Ferrand. For these comparisons, an electrolyte leakage conductivity method (LT_{50}) was used and adapted for walnut.

In all cases NG38 (*Juglans regia x Juglans nigra*) appeared particularly cold resistant.

Résumé

En Auvergne, les noyers montrent une résistance au gel qui évolue au cours de l'année (acclimatation en automne, désacclimatation au printemps). Le niveau d'endurcissement au gel varie selon la variété et la date de prélèvement. Le comportement vis-à-vis de la tolérance au froid de 7 variétés de noyer à fruit (« Chandler », « Franquette », « Fernor, Fernette », « Lara », « Pedro », « Serr ») et d'une variété hybride *Juglans regia* x *Juglans nigra* « NG38 » sont comparées sur un même lieu de culture (Centre INRA de Crouelle, Clermont-Ferrand, France). Pour cette comparaison, la méthode LT_{50} a été utilisée et adaptée au noyer.

Dans tous les cas, NG38 apparaît particulièrement résistant au gel.

INTRODUCTION

The gene pool of every species exhibits a range of variation enabling it to survive a certain degree of change in the environment (Sakai and Larcher, 1987). Low temperature represents one of the most important environmental constraints limiting plant productivity and the distribution (Kerr et al, 1997; Bravo et al., 2001).

Plants exposed to low temperatures can suffer chilling injury (at temperature above 0°C) and/or freezing injury (at subzero temperatures). The freezing tolerance of most perennial plants increases from fall to winter to prevent injury under winter conditions. This phenomenon is referred to as hardening or cold acclimation (Sakai and Larcher, 1987). Many studies have shown that the soluble carbohydrate content of plant tissues coincide with the acquisition of freezing tolerance (e.g. Dionne et al., 2001; Guinchard et al., 1997).

Selecting frost tolerant genotypes and understanding the mechanism of frost hardiness could greatly improve frost resistance for walnut.

In this study, we describe cold tolerance performances of 7 walnut fruit cultivars (Chandler, Franquette, Fernor, Fernette, Lara, Pedro, Serr) and of the hybrid *Juglans regia* x *Juglans nigra* NG38, compared in the same location, at the INRA Center of Clermont-Ferrand, in relation to changes in levels of soluble carbohydrates. For these comparisons, an electrolyte leakage conductivity method (LT50) was used and adapted for walnut.

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MATERIALS AND METHODS

Plant materials

Seven walnut fruit cultivars ("Chandler"[3], "Franquette"[1], "Fernor"[1], "Fernette"[1], "Lara"[1], "Pedro"[1], "Serr"[3]) and one hybrid *Juglans regia* x *Juglans nigra* "NG38"[2] (walnut wood cultivar) were used for this study. Trees are located near Clermont-Ferrand (INRA Clermont-Ferrand-Theix, site of Crouelle), in south-central France. Measurements were made on excised 1-year-old twigs of walnut.

[1: planted in 1994; 2: planted in 1996; 3: planted in 2000]

Frost resistance

Twig segments were used for estimating frost hardiness with an electrolyte leakage conductivity method (LT50: ie, subzero temperature causing 50% mortality: based on the methods of Wisniewski and Ashworth, 1985; Zhang and Willison, 1986). Fresh segments were washed in distilled-demonized water. Stem sections of uniform size (10 cm in length) and a moistened tissue were wrapped in aluminum foil and placed in pre-chilled Dewar flasks. Flasks were transferred to a deep freezer (-80°C). Sample temperatures were monitored using copper-constantan thermocouples inserted into the foil pouch. Samples cooled at the rate of 5-7°C/h. At any desired subzero temperature (ex. control +5°C, –5°C, -10°C, -15°C, -20°C), Dewar flasks were placed at +5°C in a refrigerator for 12-15 h to facilitate slow thawing of the samples. After thawing, internodal sections were quartered and sliced in several segments while immersed in 15ml of precooled (+5°C) distilled-deionized water into 2.5 x 20 cm test tubes. The capped tubes were placed on a gyratory shaker for a night. Initial conductivity was taken at the end of the night (12h) with an electrical conductivity meter (Conductivity hand-Held Meter LF340 with standard conductivity cell, TetraCon 325). To obtain maximum conductivity, tubes were autoclaved at 120°C for 30 min. Relative conductivity was calculated by dividing the initial conductivity value by the maximum conductivity value. After plotting the percentage of injury as a function of treatment temperatures, the temperature at which 50% injury occurs was defined as LT50.

Analysis of carbohydrate

In addition segment of twigs were frozen in liquid nitrogen, lyophilized, and their dry weight measured. Soluble sugars were then extracted from the stems with hot ethanol/water (80/20, v/v), and purified on ion-exchange resins (Bio-rad AG 1-X8 in the carbonate form, Dowex 50W in the H+ form), as described by Moing and Gaudillière (1992). Using a spectrophotometer at 340 nm, sucrose, glucose and fructose contents were determined after enzymatic assays (Boehringer, 1984)

Statistical analyses

Average and standard error values were computed for all repetitive data. Statistical analyses were conducted with Microsoft® Excel 2002.

RESULTS AND DISCUSSION

Figure 1 shows cold tolerance performances of 7 walnut fruit and 1 walnut wood cultivar. We observed significant differences between winter (maximum cold hardiness) and the other seasons. In fact, freezing tolerance is minimal during bud break. It is maximal in September-beginning October (Côme, 1992). Most plants from regions with cold winters initiate the process of cold acclimation as soon as shoot growth has ceased. For the development of maximum cold tolerance, growth cessation is usually associated with the induction of dormancy (Levitt, 1980). These differences between winter and the other seasons

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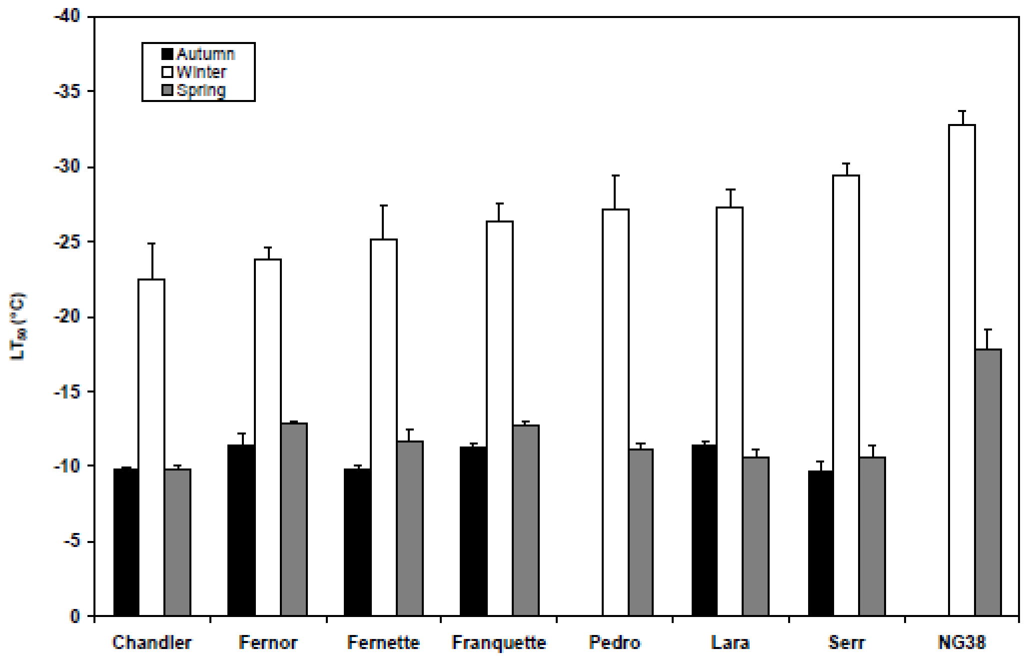

Figure 1. *Time course of cold tolerance performance of 7 walnut fruit and 1 walnut wood cultivars for 3 seasons (autumn, winter and spring). Averages +/- SE are shown (n=5).*

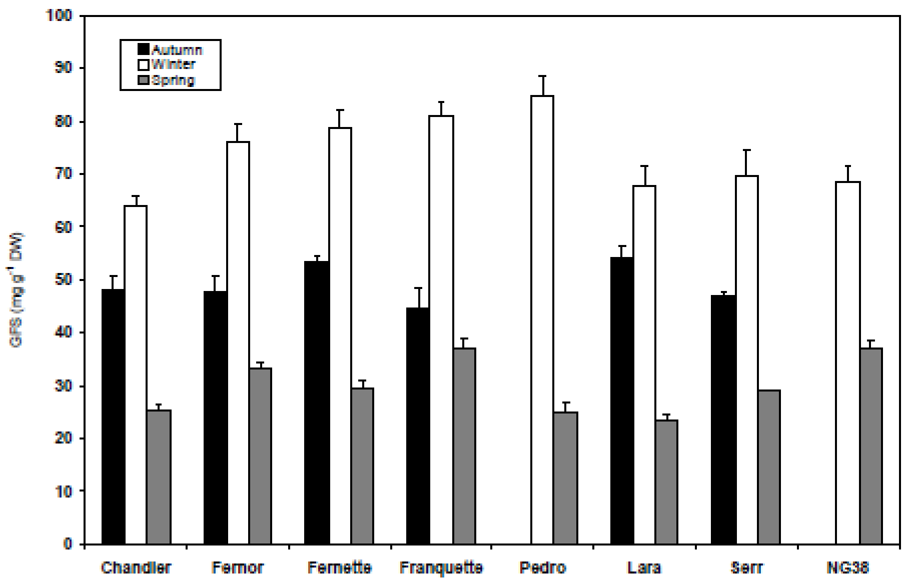

Figure 2. *Time course of sugar content (GFS: Total of Glucose + Fructose + Sucrose) of 7 walnut fruit and 1 walnut wood cultivars for 3 seasons (autumn, winter and spring). Averages +/- SE are shown (n=5).*

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have been observed between all Cvs. NG38 appeared the most cold hardening Cv. For fruit Cvs, maximum cold hardiness was not correlated to cold resistance in autumn or spring. Acclimation between autumn and winter was concomitant with the interconversion between starch and soluble sugars in parenchyma. Améglio et al. give prominence to the same interconversion. Figure 2 shows GFS concentration for the same 3 periods. Maximum GFS concentration was not strictly correlated to freezing tolerance. For example, Cv. Chandler presented the same cold tolerance in autumn and spring; nevertheless GFS concentration was more important in autumn than spring. The figure 3 shows the correlation between GFS concentration and LT50. Global tendency shows an overall correlation between the 2 variables, but the relation appeared hardly relevant in autumn. During this period, other factor must be involved. Moreover, NG 38 was most resistant than other Cvs with the same GFS concentration. Nevertheless in spring, a good correlation was observed. Cold hardiness in spring was also correlated to the bud break time (data not shown). This bud break time appeared most relevant to explain frost sensibility for production. In Clermont-Ferrand, Lara < Pedro < Chandler < Serr appeared relatively sensitive to cold temperature, contrary to Fernette < Fernor and the best adapted cultivar, Franquette.

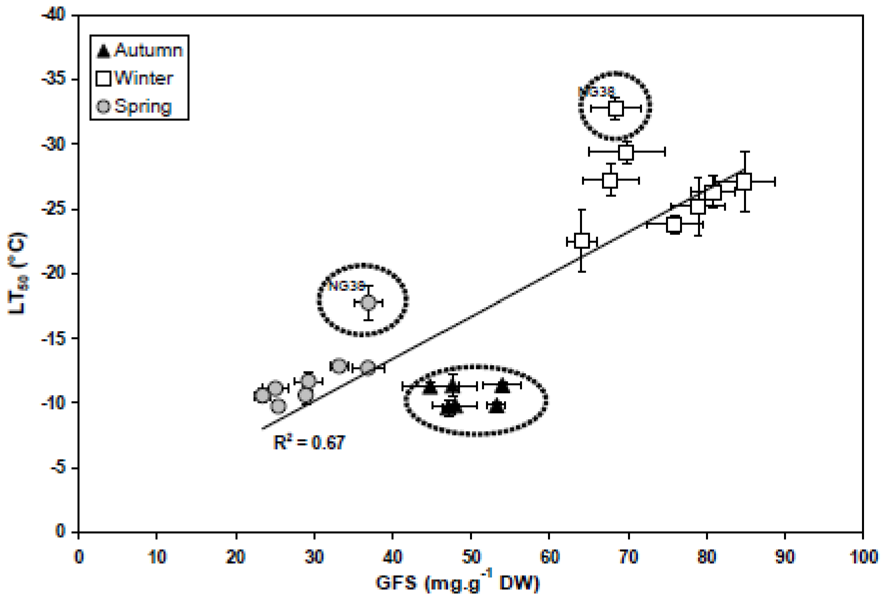

Figure 3. *Relationships between the sugar content (GFS: Total of Glucose + Fructose + Sucrose) in the parenchyma cells and the temperature where 50% of injuries occurs (LT_{50}). R^2: coefficient of determination and averages +/- SE are shown (n=5).*

This work should be considered as preliminary observations, which need further confirmation. These results are made from small portions of the tree. Small portions of the plants do not always reflect the cold hardiness of the whole plant and the response of isolated tissues and cells to low temperature may differ in some respects with that known for the whole plant system. Furthermore, the one-year-old twig is not comparable with the old twig.

Since frost resistance is a highly complex and not well-known subject in walnut, further studies also on the compounds involved in this mechanism are needed. In fact, many physiological and biological changes are apparently involved in hardiness regulation including carbohydrate metabolism, protein synthesis, gene expression, and alteration of

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membranes. Moreover, selected frost-resistant genotypes must be tested to verify their agronomic behaviour.

CONCLUSIONS

In south central France, NG38, Franquette, Fernor and to a lesser extent Fernette appeared well acclimated to cold temperature in Winter but also in Spring and Autumn. The other cultivars presented efficient cold hardening during winter, but their spring sensibility explained some damage with late frost in spring. In this condition the bud break time appeared the principal control to frost tolerance. Soluble sugar evolutions in parenchyma explained acclimation tendency, but in autumn other factors were certainly involved. In all cases NG38 (*Juglans regia* x *Juglans nigra*) appeared particularly cold resistant.

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