



Correlation of ovary and leaf spermidine and spermine content with the alternate bearing habit of olive

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Received 7 September 2004; accepted 26 January 2005

KEYWORDS

Alternate bearing;
Calcium;
Fertilization;
Fruit set;
Olea europaea L.;
Olive;
Polyamines;
Potassium;
Spermidine;
Spermine

Summary

In order to investigate the possible role of the polyamines spermidine and spermine, in the alternate bearing habit of the olive, a comparative analysis of their content in vegetative and reproductive organs during the flowering period of the 'on' and 'off' years, was conducted. Samples of flowers, ovaries and leaves from two cultivars, the biennial bearer 'Chondrolia Chalkidikis' and the regular cropping 'Koroneiki' were used and the free and conjugated forms of polyamines were detected. In addition, the concentrations of K^+ and Ca^{2+} were determined in all samples. Spermidine conjugates were predominant in all samples, while free spermine was not detectable. Conjugated forms of both spermidine and spermine were accumulated in ovaries, rather than in other floral tissues (corolla and stamens). They also, accumulated in significantly higher concentrations in ovaries and leaves of both cultivars, during the 'on' compared with the 'off' year, and in much higher concentrations, during the 'on' year, in ovaries of the strong alternate bearer 'Chondrolia Chalkidikis' than in 'Koroneiki'. Free spermidine was detected in ovaries and leaves of both cultivars, showing a decline during ovule fertilization in both years. Such a decline was not always recorded for the conjugated forms, depending on the organ studied and the flower load. A relation between endogenous spermidine and spermine with the K^+ or Ca^{2+} composition was not established.

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Abbreviations: AFB, after full bloom; PA, polyamine; Spd, spermidine; Spm, spermine

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Introduction

Most olive cultivars exhibit a biennial production cycle (alternate bearing), which is one of the most troublesome problems of the olive industry. It has been attributed to the competition for assimilates, due to the partial overlap among bud differentiation, inflorescence growth, fruit set, fruit growth and shoot growth (Cuevas et al., 1995; Proietti and Tombesi, 1996). Horticultural practices including thinning, girdling, pruning and irrigation reduce the severity of biennial bearing, but are not effective enough to overcome it (Lavee et al., 1986). Variations in floral quality are related to flower load and may reinforce or alleviate alternate bearing. A better understanding of the floral biology of the olive in general and of specific cultivars in particular might be of major importance in controlling and regulating the bearing habit of this species.

Attempts have been made to study the role of various plant growth regulating compounds in reproductive processes. Among them polyamines (PAs) have been found to be promising substances in controlling fruit set (Kakkar and Rai, 1993; Faust and Wang, 1992). Application of PAs increased fruit yield in apple (Costa and Bagni, 1983), although the results were sometimes controversial. An increase in fruit set was especially significant in self-incompatible olive cultivars, when putrescine was sprayed on flowers at high concentrations (Rugini and Mencuccini, 1985).

Polyamines are organic polycations, implicated in a variety of physiological events, among them floral development and stress tolerance mechanisms (Evans and Malmberg, 1989; Martin-Tanguy, 1997; Bagni and Tassoni, 2001). In these roles, PAs interfere with plant hormones and other compounds (Carbonell and Navarro, 1989; Kakkar and Rai, 1993; Russos et al., 2002). There is evidence associating PAs, especially their conjugates, with the development of reproductive organs and ovule fertilization; therefore they are considered as 'markers of fertility', although their mode of action has not yet been clarified (Martin-Tanguy, 1997; Bagni and Tassoni, 2001; Guo et al., 2003). Some of their biological functions may be explained by their role as organic carriers of positive charge, thus, their relationships with inorganic cations, mainly K^+ and Ca^{2+} , have been studied (De Agazio et al., 1988; Minocha et al., 2000).

In view of the multiple roles attributed to PAs, it should be informative to investigate their possible involvement in the reproductive physiology and, particularly, in mechanisms leading to the alternate bearing of the olive tree, as was previously done in pistachio (Russos et al., 2004).

The aim of this study was to investigate the role of the endogenous PAs spermidine (tri-amine) and spermine (tetra-amine) in olive ovaries and leaves at bloom and a few days after, when the fertilization of the ovules has been completed, in the 'on' and 'off' year, in trees of two cultivars differing in their alternate bearing habit. Furthermore, because PAs are considered to act as cations (De Agazio et al., 1988), their possible relation with the K^+ and Ca^{2+} content of ovaries and leaves was investigated during the same period.

Materials and methods

Plant material

Samples were collected in two successive years, 1998 and 1999 being the 'on' and the 'off' years, respectively, from 30-year-old olive (*Olea europaea* L.) trees at the orchard of the University farm (lat. $40^{\circ}37'N$). Two cultivars, 'Chondrolia Chalkidikis' (Ch. Chalkidikis) and 'Koroneiki', differing in fruit size and in alternate bearing behaviour, were studied. The former is a large-fruited (table olive) cultivar, with a distinct 2-year cycle of fruit bearing, while the latter is a small-fruited (oil olive) cultivar, bearing a regular crop every single year. It should be noted that 'Koroneiki' does show a biennial flower load alteration, but in a very narrow range, its productivity through the years being constant. A total of 12 experimental trees (3 replications with 4 trees per replication) of each cultivar were selected on the basis of their synchronous bearing phase. Thus, samples of 1998 and 1999 were collected from 'on' and 'off' shoots, respectively.

The experimental materials were: (a) ovaries from perfect flowers at full bloom and fertilized ovaries 14 days after full bloom (AFB), for the determination of their growth rate, and (b) perfect flowers at the green balloon stage and their ovaries, and fully expanded leaves and ovaries at full bloom (bloom) and (c) fertilized ovaries (fert.) 2–3 weeks AFB, for PA determination. The fertilized ovaries were collected when most of the fruitlet drop had taken place and before embryo growth initiation (Rapoport and Rallo, 1991; Cuevas et al., 1995), i.e. 14 or 21 d AFB for 1998 and 1999, respectively. Full bloom (80% open flowers) was recorded on May 15 and May 27, for 'Ch. Chalkidikis' and 'Koroneiki', respectively, in 1998 and on May 24 and May 27, for 'Ch. Chalkidikis' and 'Koroneiki', respectively, in 1999. Samples were immediately transferred, in dry ice, to the

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