Phenylalanine Ammonia-lyase, Peroxidase, Pyruvic Acid and Total Phenolics Variations in Onion Bulbs During Long-term Storage

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Phenylalanine ammonia-lyase (PAL) and peroxidase (POD) enzyme activities, total phenolics and pyruvic acid (PA) (as pungency indicator) were measured in onion bulb tissues (c.v. Rouge Amposta) during storage at 4 and 20 °C. After 2 wk, PAL activity increased at 4 °C, but decreased at 20 °C, and after 4 wk, PAL activity at 20 °C was higher than activity observed at 4 °C. Peroxidase activity was high during the first weeks of storage, but at 4 °C this high activity lasted longer and a decrease was noted at both temperatures, coinciding with sprouting appearance. Total phenolics, which rose during the first 12 wk but fell during last weeks of storage, appeared to be linked to PAL activity. An inverse relationship was observed between phenolic content and the amount of sprouting development of bulbs. Pyruvic acid production appeared to be influenced by temperature, but in the last period of storage, the effect of temperature on PA production was less. The data demonstrate that PAL activity, which is linked to phenolic metabolism, and POD are highly involved in the sprouting of onion bulbs and these two enzymatic activities are much influenced by low temperature.

Keywords: PAL; POD; phenolics; storage; onion

Introduction

Onion bulbs (Allium cepa L) are one of the oldest vegetable crops known to man. A comprehensive account of this vegetable as well as storage aspects are contained in the literature (1,2). Onions are the major vegetable crop in Algeria, being valued mainly for their flavour and used for cooking. Classical and modern technologies have greatly increased the shelf-life and quality of vegetable products including onions which may often sprout or rot by storing them at low temperatures (1,3). Nevertheless, during long-term storage, important biochemical (and physiological) changes occur in bulbs due to sprouting affected by temperature. Phenylalanine ammonia-lyase (PAL, EC 4. 3. 1. 5) is the entry-point enzyme of the phenyl propanoid biosynthesis pathway, and its presence has been demonstrated in all higher plants. It catalyses the elimination of ammonia from L-phenylalanine to give trans-cinnamate, this being the "first committed step for the biosynthesis of plant "specific phenylpropanoid derivatives such as phenolics (4). Activity of PAL was found to vary greatly with stage of plant development and with various stresses (5). Peroxidases (POD, EC 1. 11. 1. 7) are widely distributed in living organisms. The primary function of the POD is to oxidize molecules at the expense of hydrogen peroxide. Its possible involvement in the dormancy of onion bulbs was reported by Benkeblia and Selselet-Attou (6) and inverse relationships have been established between the growth rate and peroxidase activity (7). Pyruvic acid is produced by allinase hydrolysis of a group of flavour-precursor S-alk(en)yl-l-cysteine sulphoxides in onion tissues when they are chopped or macerated (8), and many sulphur-volatiles and ammonia also are produced during this reaction (9). According to Wall and Corgan (10), a high correlation between enzymatically produced PA and pungency perception was noted. Phenolic compounds in onions are mainly formed by anthocyanins (11) and flavonoids (12). These constituents may be involved in the response of onion tissues to cold stress and in the degree of dormancy of the buds of bulbs (6). For onion bulbs, however, information is lacking on the changes in enzyme activities such as PAL and POD, and of pyruvic acid and phenolics as related to sprouting during storage and as affected by low temperature.

The present study was carried out to assess the variations of PAL and POD activity, phenolic and pyruvic acid during storage of onion bulbs, and to determine the relationships between those changes and sprouting.

Materials and Methods

Onions

'Rouge Amposta' onion bulbs were grown in Mascara area (Algeria), harvested in August and dried in the field.

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for 2 wk. The bulbs were then sorted for uniformity and absence of defects and packed in commercial plastic trays of 12 kg.

Storage
Onion bulbs were packed in commercial plastic (PVC) trays of 12 kg, and stored for 22 wk at 4 °C and 20 °C at 90% and 65% RH, respectively. During storage, onion bulbs were scored weekly for sprouting by emergence of green leaves from the neck of the bulb.

Assay of phenylalanine ammonia-lyase (PAL) activity
Activity of PAL was determined on nine bulbs by the production of trans-cinnamic acid from l-phenylalanine over 1 h at 30 °C, and was measured spectrophotometrically (PUY UNICAM SP 9000 model) by the absorbance change at 290 nm (13). Internal – inner scales + sprout – fresh tissues (10 g of each bulb) were homogenized in 100 mL acetone and the insoluble residue filtered and dried under vacuum. It was extracted at 4 °C for 1 h by gentle stirring with 50 mL of extraction buffer which contained 38 g/L sodium borate (pH 8.8), 0.39 g/L β-mercaptoethanol, 0.58 g/L EDTA (ethylenediaminetetraacetate) and PVPP (polyvinylpolypyrrolidone) at 1 g/100 g of the onion fresh weight. After 1 h of extraction, the solution was filtered through one layer nylon cloth and centrifuged at 20 000 × g at 4 °C for 15 min and the supernatant collected. The reaction mixture for the assay contained 1.5 mL of sodium borate buffer (pH 8.8), 0.5 mL of supernatant and 1 mL of 2.5 g/L l-phenylalanine added after 10 min of preincubation. One unit of PAL activity equals the amount of PAL that produces 1 μmol of cinnamic acid in 1 h.

Assay of peroxidase (POD) activity
Activity of POD was determined on nine bulbs according to the method of Guené and Bayindirh (14). Internal (same as above) fresh tissues (10 g of each bulb) were mixed with 50 mL of phosphate buffer (pH 7.0) and the supernatant was removed for peroxidase assay. Activity was determined by measuring the absorbance of 0.001/min.

Pyruvic acid (PA) analysis
Pyruvic acid analysis was performed according to Schwimmer and Weston (15) on nine bulbs. Internal (same as above) fresh tissues (50 g of each bulb) were quickly sliced into 150 mL of diluted TCA (trichoroacetic acid, water; 1:20) to inactivate the alliinase enzyme, and blended in Vorwerk blender at maximum speed for 3 min. After 1 h maceration, the mixture was filtered and the filtrate was diluted (1:10) and analysed for PA. Each reaction tube contained 1 mL of diluted filtrate, 1 mL of distilled water and 1 mL of 2.4 dinitrophenylhydrazine (125 mg/L of DNPH in 73 g/L HCl). Reaction tubes were vortexed and placed in a water bath (37 °C) for 10 min. After the incubation period, 5 mL of 24 g/L NaOH was added and tubes were vortexed for 5 min. Pyruvic acid was measured using spectrophotometer (PUY Unicam SP 9000 model) at 490 nm, and a standard curve was obtained from pure pyruvic acid (Sigma Chemical Co., St Louis, U.S.A.).

Total phenolics determination
Total phenolics were extracted as described by Brenes et al. (16), and determined on nine bulbs according to the AOAC method (17). Samples of internal (same as above) fresh tissues (10 g of each bulb) were mixed with 80 mL of aqueous methanol (800 mL/L) and 20 mL of 5 g/L metabisulphite, homogenized for 30 s and left for 15 min at 4 °C. The homogenate was filtered on Büchner prior to analysis. Total phenolics of extracts were quantified colorimetrically at 730 nm with a PUY Unicam spectrophotometer (SP 9000 model), using chlorogenic acid (Sigma Chemical Co.) as a standard.

Statistical analysis
All determinations were carried out in triplicate (nine bulbs × three times; n being defined as one mean value of nine bulbs) and expressed on a fresh weight basis. The experiment was repeated in two successive harvesting seasons (1995, 1996). The data from the different sample sets of temperature were compared by Student test (t) to determine the effect of temperature on PAL and POD activities, variation of PA and phenolic contents using XLStat Pro (SL Stat, Paris, France) software (18).

Results and Discussion
Phenylalanine ammonia-lyase (PAL) activity
During the first 2 wk of storage at 4 °C, PAL activity increased to 5.84 units/100 g of fresh weight, then decreased to 2.71 units by 10 wk, finally increasing to 4.26 units during the last 12 wk of storage (Fig. 1). At 20 °C, PAL activity decreased to 5.33 units during the first 4 wk, increased to 6.36 units in the next 4 wk and then decreased to 5.27 units during the last 14 wk of storage. The effect of low temperature stress on PAL-induced activity has been reported by several authors (5,19,20). An increase in PAL activity and anthocyanin accumulation was reported for apples (21) and pomegranates (22), and changes were related to the fruit colour during their ripening process.

Peroxidase (POD) activity
At 4 °C, POD activity showed a similar pattern, although at different timescales. Activity increased 110 units/100 g
Phenylalanine ammonia-lyase variation in onion bulb tissues during storage. O, 4 °C; △, 20 °C. Bars represent s,

Peroxidase changes in onion bulb tissues during storage. For key see Fig. 1

Pyruvic acid changes in onion bulb tissues during storage. For key see Fig. 1

Total phenolic changes in onion bulb tissues during storage. For key see Fig. 1

of fresh weight during the first 10 wk, decreased 81.7 units between 10 and 18 wk, and increased 100 units during the last 4 wk of storage. At 20 °C, POD activity increased to 140 units during the first 6 wk, decreased to 106.7 units until week 14 and then increased to 125 units during the last 6 wk of storage (Fig. 2). The first decrease in POD activity at either temperature coincided with the onset of bulb sprouting.

The catalytic role of POD has been widely studied, especially on indol acetic acid (IAA), which is considered an effective promoter of growth of plant tissues such as the inner bud of bulbs (23,24). Croci et al. (25) reported an inverse relationship between the growth of the inner sprout of garlic cloves and POD activity in tissues after 50 d at 18 °C in darkness.

Pyruvic acid (PA)

The PA content of onion tissue showed similar changes at 4 °C and 20 °C during the first 10 wk, increasing to 42.5 and 52.5 μmol/g of fresh weight, respectively, followed by a decrease to 32.5 and 42.5 μmol (Fig. 3). After 10 wk at 4 °C, PA increased to 42.5 μmol between 10 and 16 wk, and decreased to 32.5 μmol between 16 and 22 wk, whereas at 20 °C, a decrease of PA production to 37.5 μmol was observed between 10 and 16 wk, followed by an increase to 52.5 μmol between 16 and 22 wk of storage.

The same irregular patterns of PA formed in onion tissues were reported by Kopsell and Randle (26) in five cultivars of onion. Ceci et al. (27) noted a similar variation in PA concentration in garlic stored for 300 d at a temperature ranging from 6 °C to 32 °C. Nevertheless, PA content of onions depended on several factors such as dry matter and sugar content (28), cultivars (26), maturity (29,30) and sulphur nutrition (28,31). The PA content also varied with changes in dry matter during storage, caused by weight loss and dehydration of tissues, and these explain the increase in PA observed at 20 °C during the last weeks of storage.

Total phenolics

Variation in phenolics was rather regular during storage at both temperatures. At 20 °C, phenolics increased 0.1 mg/wk between 0 and 10 wk, followed by a decrease of 0.45 mg/wk (Fig. 4). In contrast, at 4 °C, phenolic content decreased 0.52 mg/wk between 0 and 10 wk and then increased slightly 0.06 mg/wk. In both cases, the rates of the increase (0.1 mg and 0.06 mg per week) and the decrease (0.45 mg and 0.52 mg per week) were similar, and the increase in PAL activity caused the rise in total phenolic content in tissues. Ke and Saltveit (32) reported a positive relationship between PAL activity and total phenolics in lettuce and the same observation was noted by Riov et al. (33) in
citrus fruit peel. In the same way, Tan (20) reported a similar relationship between PAL activity and anthocyanins in whole apples, and Benkeblia (11) noted a decrease in anthocyanins in stored red onions at 20°C.

**Sprouting**

Sprouting of bulbs followed a regular development during storage at both temperatures (Fig. 5). At 20°C, sprouting occurred after 4 wk and increased by 3% per wk reaching 25% after 14 wk, increased by 2.5% per week, finally reaching 25% sprout. The sprouting of onion bulbs has been widely investigated (1,2), and it appears that sprout development is dependent on several conditions including environmental factors such as temperature (34,35), and other secondary factors, such as cultivars (35).

**Statistical analysis**

Statistical analysis of data showed a highly significant effect (at $P < 0.05$) of temperature on the variation of PAL and POD activity and PA content during storage. In contrast, no significant effect of temperature on phenolic variation was detected.

**Conclusion**

This study indicated that PAL activity induced synthesis of phenolics, which are probably involved in the sprouting process. In contrast, POD activity is probably linked to specific metabolisms which could affect sprout growth and development. Pyruvic acid, a pungency indicator, was much influenced by storage temperature and probably affected by water content of the bulb tissues where it is formed. Further investigation is necessary to determine the relationship between PAL activity and the chemical nature of phenolic compounds involved during sprouting, and also to determine the cellular localization of the compounds in order to identify the exact type of PAL-phenolic reaction. Similarly, it will be necessary to characterize the biochemical nature of PAL and POD enzymes in onion tissues to facilitate studies on their reactions.

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**References**


