Effects of Low Temperatures on Changes in Oligosaccharides, Phenolics and Peroxidase in Inner Bud of Onion \textit{Allium cepa} L. During Break of Dormancy


The effects of low temperatures on the break of dormancy of onion bulbs and changes in oligosaccharides, phenolics and peroxidase activity were examined. Cold treatment at 9°C was more effective on break of dormancy than at 0°C, but in both cases cold temperatures caused a rapid break of dormancy. Changes in oligosaccharides and glucose were linked to sprouting stages, with higher concentrations at the onset of sprouting for cold treatments of 9°C for 3 weeks and 0°C for 2 weeks; for the other treatments (9°C for 2 weeks and 0°C for 3 weeks) the high concentrations were reached after sprouting. There was an inverse relation between sprouting on the one hand, and phenolics and peroxidase activity on the other hand. These results emphasized a positive effect of low temperature on breaking dormancy of onion bulbs and the negative role of phenolics and peroxidase on inner bud development.

Key words: biochemical changes, chilling, sprouting.

Introduction

Dormancy of onion bulbs, which is defined as a temporary suspension of visible growth of the meristematic tissues of the sprouts, has a major impact on the storage of bulbs, influencing such processes as vegetable growth (Lang, 1987; Lang et al., 1987; Juntilla, 1988).

One of the most popular approaches in the study of dormancy has been to study the physiological basis of dormancy in sprout during subsequent exposure to rest-breaking treatments at low temperature (Dennis, 1987). During the rest period, matured onion bulbs fail to sprout under conditions ordinarily favourable for growth. Abdallah & Manns (1963) demonstrated that the rest period eventually disappeared with time at all storage temperatures, but did so more quickly at some temperatures than others. Gradual changes in the biochemistry of the bulbs throughout the dormancy period were observed, and low temperatures caused compositional changes and physiological disorders in tissues (Weichmann, 1987). Kato (1966) observed a correlation between carbohydrate metabolism and dormancy of onions.

The role of phenolics in the dormancy process and sprouting of onions is unknown. Cvirkova et al. (1994) reported an inhibiting action of phenolic compounds in potato tuber buds and their concentration decreases during the break of dormancy in potato tissues. The role of peroxidase in auxin degradation is well established (Greppin et al., 1986), but its involve-
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ment in dormancy phenomena and sprouting is not very clear in onion bulbs. Poovaiah et al. (1972) observed a higher peroxidase activity in onions with short dormancy than in onions with long dormancy. Experiments have proved a relationship between the hormonal changes and the physiological status of bulbs during their different stages, which are influenced by several factors, e.g. hormone level (Thomas, 1969; Thomas & Isenberg, 1972) and temperature (Bertaud, 1990; Miedema & Kamminga, 1994; Miedema, 1994).

The present investigation was carried out to assess the effects of low temperatures on breaking dormancy of onion bulbs and the biochemical changes in the inner buds during sprouting.

Materials and methods

Onions

‘Rouge Amposta’ onion bulbs were grown in the Mascara area (Algeria), harvested in August and dried in the field for 2 weeks. The bulbs were then sorted for uniformity and absence of defects and packed in commercial plastic trays of 12 kg.

Low-temperature treatments

Bulbs were treated at two temperatures and cooling durations: 0°C for 2 weeks, 0°C for 3 weeks, 9°C for 2 weeks and 9°C for 3 weeks. Thereafter, bulbs were stored at 18°C and 70% relative humidity (RH). Sprouting was counted from the first week of cold treatment. Thirty bulbs were cut longitudinally twice a week to check for sprouting (appearance of first green internal leaves), and soluble sugars, total phenolics and peroxidase activity were assessed simultaneously on nine bulbs.

Analysis of soluble sugars

Glucose, fructose and sucrose were determined by high-performance liquid chromatography (HPLC; Doyon et al., 1991). Samples of 5 g of inner bud freeze-dried tissues were homogenized in 50 ml of water. The homogenate was heated for 30 min in a boiling water bath. After cooling, the homogenate was centrifuged for 15 min at 25000 g and the supernatant was filtered on a 0.25 μm Millipore filter (Dynagard, Merck). The sugars were separated by HPLC using a Varian 5000 model fitted with a Polyspher CH-CA column (300 × 7.8 mm; Merck) set at 80°C and a differential refractometer detector (Knauer model). The mobile phase was DDI water at a flow rate of 0.5 ml min⁻¹. Sugars were identified and quantified by comparison with authentic samples (Sigma Chemical Co., St Louis, USA).

Total phenolics

Total phenolics were extracted as described by Brenes et al. (1992) and determined according to the AOAC method (Horwitz, 1985). Samples of inner bud fresh tissues (10 g) were mixed with 80 ml of 80% methanol with 0.5% metabisulfite, homogenized 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) and using chlorogenic acid as a standard.

Assay of peroxidase activity

Peroxidase activity was determined according to the method of Günes & Bayindirh (1993). Inner bud fresh tissues (5 g) were mixed with 50 ml of 0.05 M phosphate buffer, pH 7.0, and blended for 5 min in a Vorwerk blender at minimal speed. The homogenate was centrifuged for 15 min at 20000 g and the supernatant was used for peroxidase assay. Peroxidase activity was determined by measuring the colour development at 430 nm with a Puy Unicam spectrophotometer (SP 9000 model). One millilitre of extract was mixed with 1 ml of 0.5% gaiacol, 1 ml of 0.5% hydrogen peroxide and 18 ml of phosphate buffer, pH 6.5. One unit of activity was defined as a change in absorbance of 0.001 min⁻¹.

All experiments were triplicated and they were repeated in two successive harvesting seasons (1995 and 1996).

Results and discussion

Break of dormancy

The break of dormancy was affected more by the 9°C than the 0°C treatment (Fig. 1). At 9°C, there was a 100% break of dormancy after 4 weeks for bulbs with a 3-week cold treatment, and after 5 weeks for those with a 2-week cold treatment. With cold treatment at 0°C, total sprouting was observed later: after 6 weeks for the 3-week cold treatment and after 7 weeks for the 2-week cold treatment. In comparison, only 20% of the control bulbs started sprouting after 8 weeks.

Aguettaz et al. (1992) reported 90–100% sprouting of bulblets of *Lilium speciosum* regenerated *in vitro* after 6 weeks of cold treatment at 0°C. In general, the response of dormant organs to cold treatments was noted between 4 and 12°C (Dennis, 1987).

Effects of temperature on dormancy of onion bulbs and some other vegetables have been reported by several authors (Courduroux et al., 1972; Niimi et al., 1988). These effects depend mainly on the duration of cold treatment and sensitivity of some vegetables to chilling (Lyons & Breidenbach, 1987). According to
Fig. 1. Effects of cold treatments on break of dormancy of inner buds in onion bulbs. (♦ = Control; ■ = 0°C 2 weeks; △ = 0°C 3 weeks; × = 9°C 2 weeks; ○ = 9°C 3 weeks)

Le Nard (1982), some flower bulbs were sensitive to chilling while others needed higher temperatures for sprouting.

Changes in soluble sugars
Changes in soluble sugars and glucose were similar (Figs. 2 and 3). High concentrations were observed after 3 weeks for both cold treatment at 9°C for 3 weeks and at 0°C for 2 weeks. However, with cold treatment at 9°C for 2 weeks and at 0°C for 3 weeks, high levels of soluble sugars and glucose were observed after 4 weeks. The concentration of soluble sugars for the control samples peaked after 6 weeks.

It appears that the hydrolysis of fructans and/or oligosaccharides and their accumulation for sprout metabolism were reduced by cold temperatures. Cold treatment at 9°C for 3 weeks and at 0°C for 2 weeks displayed, 1 week earlier, the same effect as 9°C for 2 weeks and 0°C for 3 weeks in metabolism induction of oligosaccharides and glucose in inner buds of onions during break of dormancy.

Changes in soluble sugars in bulbs during dormancy and sprouting have been reported by some authors but changes in these compounds in inner buds during breaking dormancy have not been extensively studied (Rutherford, 1981). Pak et al. (1995) noted results similar to those of this study, with high concentrations of glucose but low concentrations of fructans in bulbs after 5 weeks of storage at 16°C. Peaks of glucose and fructose preceded an increase in the mitotic index in sprouted buds. According to Rutherford (1981), metabolism of oligosaccharides was extremely influenced by temperature in onion bulbs during sprouting.

Total phenolics
In cold-treated onion bulbs, total phenolics in inner buds decreased after 1 week and reached 10 mg 100 g⁻¹ FW after 4 weeks with 9°C treatment for both chilling durations. However, with 0°C treatment, an
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An increase from 17 to 20 mg 100 g\(^{-1}\) FW was observed during the first and second weeks, then there was a decrease to 12 mg 100 g\(^{-1}\) FW for both cold treatment durations (Fig. 4). In the inner buds of the control samples, an increase from 17 to 20 mg 100 g\(^{-1}\) FW was observed after 5 weeks and a decrease to 15 mg 100 g\(^{-1}\) FW after 7 weeks when bulb began to sprout.

It appears that high levels of phenolics inhibit sprouting and low temperature triggers a signal to decrease these compounds, promoting a break of dormancy. Relations between phenolics and break of dormancy are not yet clearly known. However, results similar to those of this study were observed in inner buds of potatoes during the break of dormancy (Cvirkova et al., 1994). The sprouting process may induce a cellular oxidizing metabolism, and the presence of phenolics could inhibit or slow down sprouting because of their affinity towards oxygen. According to Hasegawa et al. (1991), an infiltration of water in the inner buds of some bulbs induces a rapid break of dormancy.

**Peroxidase activity**

Peroxidase activity in the inner buds appears to follow the same pattern as phenolics, with a decrease in all chilled bulbs (Fig. 5). A decrease in peroxidase activity (48%) was observed after 3 weeks in the inner buds of bulbs treated at 9°C for 3 weeks. After 4 weeks, decreases of 35%, 25% and 25% were observed for treatment at 9°C for 2 weeks, 0°C for 2 weeks and 0°C for 3 weeks, respectively. In the control samples, a decrease of 25% was noted after only 8 weeks. This decrease in peroxidase activity coincides with the decrease in phenolics and the onset of sprouting. The role of peroxidase has been widely studied (Penel et al., 1992), especially its catalytic activity on indol acetic acid (IAA), which is considered an effective promoter of growth and development of tissues and organs (Varner & Ho, 1976; Nissen, 1985; Goldberg et al., 1986). According to some authors, however, IAA is not the main factor but only one of the factors promoting sprouting; benzyladenin (cytokinin) is another factor (Thomas, 1969; Rutherford, 1981).

In conclusion, the results of this investigation indicate that cold treatment, particularly at intermediate temperature (9°C), induces a break of dormancy of the inner buds of onion bulbs. Low temperatures cause a decrease in phenolics and peroxidase activity, which are probably responsible for extending the dormancy status of onion bulbs. It was established that the break of dormancy was a physiological phenomenon, and cold treatment of ‘rest’ organs induces a signal triggering biochemical and other physiological modifications of sprouting. Further investigations are necessary to define the exact role of low temperatures and to establish the nature of a possible chill-induced signal through molecular biological studies.

**References**


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