REVISTA ESPAÑOLA DE FISIOLOGIA, 33, 143-148. 1977

Catalase and Peroxidase Activities, Chlorophyll and Proteins During Storage of Pea Plants at Chilling Temperatures

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(Received on December 10, 1976)

L. A. DEL RIO, M. GOMEZ and J. LOPEZ-GORGE. *Catalase and Peroxidase Activities, Chlorophyll and Proteins During Storage of Pea Plants at Chilling Temperatures.* Rev. esp. Fisiol., 33, 143-148. 1977.

The effect of chilling temperatures on the catalase and peroxidase activities, soluble proteins and chlorophyll contents of excised organs of *Pisum sativum* plants has been studied. In leaf and stem tissues, storage at 0° C did not bring about any statistically significant variation in the levels of heme enzymes, proteins and chlorophyll during four days. On the contrary, in root tissues catalase activity experimented a statistically significant depression after the onset of cold storage and during the whole treatment, whereas the other parameters remained nearly constant.

Results obtained showed the suitability of storing plant material at 0° C for the stabilization of catalase, peroxidase and chlorophyll in leaves and stems, as well as of peroxidase activity in roots.

The enhancement of activity of some enzymes during plant senescence has been demonstrated (19). Peroxidase and catalase undergo increases in activity during ripening of mangoes (13), and peroxidase shows a threefold increment in banana and tomato (5, 7). An increase in the level of the peroxidase isozymes has also been described in pear (4).

In excised plant materials, apart from enzymatic changes due to degenerative tissue injury, induced metabolic and physiological changes occur which may be related to cellular differentiation *in situ* (20). For this reason, lowered storage temperatures have been used for many years as an effective means of extending the postharvest life of fruit and vegetables (10).

However, some plant species are sensitive to chilling and, as a result, the metabolic system of the cell is disrupted bringing about imbalances in metabolism, accumulation of toxic compounds and increased cell permeability (12). Many enzyme systems isolated from chilling-injured tissue have shown an altered activity. Increases have been reported in

the catalase activity of sensitive bananas as a result of chilling treatment (14).

In contrast with the numerous reports of postharvest-induced enzymic changes in commercial ripening fruits, little is known about catalase and peroxidase activity changes in different organs of other plants which are widely used in biochemical research. Data concerning the activity of these enzymes in stored plant tissues may be useful when the enzymatic activity of a large number of plants has to be assayed, as well as for metabolic studies and for isolation and purification purposes. This report deals with catalase and peroxidase activities, chlorophyll and proteins of detached leaves, stems and roots of pea during storage at chilling temperature.

Materials and Methods

Plant material. Pea seeds (*Pisum sativum* L., var. Lincoln) were thoroughly washed with tap water and sown in sterile sand in the greenhouse. After germination the plants were grown in hydroponic culture using a continuous flow system (8). The experimental arrangement followed was a 5 × 5 latin square with 25 pots, each pot containing three plants. Five lots were prepared, each one including a pot from each file, so that the greenhouse gradients could be compensated.

The nutrient solution had the following composition in meq/l: NO_3^-, 15,21; $H_2PO_4^-$, 0.53; SO_4^{2-}, 3.76; K^+, 5.21; Ca^{2+}, 10.42; Mg^{2+}, 3.91 and in p.p.m.: Fe, 5; Mn, 0.5; B, 0.5; Cu, 0.05; Zn, 0.05. The source of iron was hydrogen ferric ethylenediamine di-(*O*-hydroxyphenylacetate) (Geigy Chemical Co.). Reagent grade chemicals were used for macronutrients and analytical grade for micronutrients.

Forty-day-old plants were employed for the assays. Leaves, stems and roots were detached and washed separately with abundant tap water first and then with distilled water. No nodules were detected in the roots. Plant material was dried on filter paper and stored, protected from light, at an average temperature of 0° C. Assays were run at the zero time of storage and after 1, 2, 3 and 4 days in the cold. A lot of 15 plants was used for each of the five storage times and all the lots were assayed by duplicate. The whole experiment was repeated three times using plants of the same age. The analytical results obtained were tested statistically for significance at 0.1 %, 1 % and 5 % levels of probability.

Enzyme preparation. Leaves, stems and roots (3 g) were cut into short segments and blended in 19 ml of 50 mM Tris-HCl buffer, pH 7.5, 1 mM DTT. Homogenates were filtered through five layers of nylon cloth and aliquots were taken for chlorophyll determination. The remaining sample was centrifuged at 1,500 *g* for 15 min and the supernatant was used for protein and enzymatic assays. All operations were performed at 4° C. Prior to the catalase and peroxidase determinations plant extracts were preincubated with Triton X-100, 0.65 % final concentration, for 1 min.

Assays. Catalase was determined polarographically by the Clark electrode method as modified by del Río *et al.* (3). The assay mixture consisted of 50 mM phosphate buffer, pH 7.0, and 33.5 mM hydrogen peroxide in 3.10 ml. The reaction mixture was started by adding 50 µl of a suitable dilution of enzyme extract and the oxygen released was measured at 25° C. Catalase activity in µmoles O_2/min was calculated as described (3).

Peroxidase was determined using *o*-dianisidine as the H-donor. The assay mixture contained 0.33 mM *o*-dianisidine, 1.0 mM H_2O_2 and 67 mM phosphate buffer, pH 6.0, in 3 ml. The reaction mixture was started by the addition of 50 µl of a dilution of enzyme extract and

the appearance of oxidized dianisidine was measured at 460 nm and 25° C. Reaction rates were calculated from the initial slope; a unit being defined as the amount of enzyme causing a change of one absorbance unit per min.

Protein was estimated by the method of LOWRY *et al.* (11) as modified by POTTY (18) for the presence of phenols and pectins. Chlorophyll was determined as described by ARNON (1).

Results and Discussion

The effect of storing detached leaves, stems and roots of peas at chilling temperatures on the catalase activity is shown in figure 1. Twenty four hours after the onset of the cold treatment catalase increased in leaves and stems, the increase being more intense in the former organ. Further storage times did not have any apparent effect on the enzymic activity. The significance of the differences was tested by contrasting the values of catalase activity obtained immediately after the plant detachment (zero time of storage) with the values after different storage times. It was found that the increase in leaf catalase was not significantly different at the 5 % level; statistically significant increases were only observed at the 10 % level. Likewise, the values of catalase activity in stems were not found to be statistically significant at the 5 % level and the activity remained more o less constant during the whole treatment.

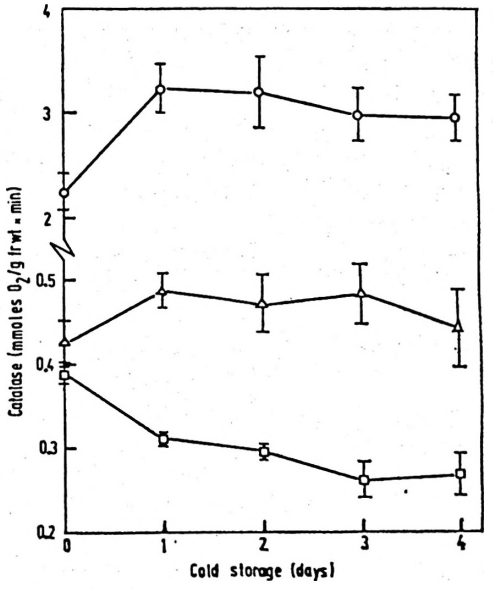

Fig. 1. *Influence of storage of leaves, stems and roots of pea plants at low temperature on catalase activity.*
Leaves (O), stems (△) and roots (□) from *Pisum sativum* L., prepared as described in Materials and Methods, were stored at 0° C during different times. Catalase was determined as described in the text. Each point with vertical bars represents means ± SEM of six samples.

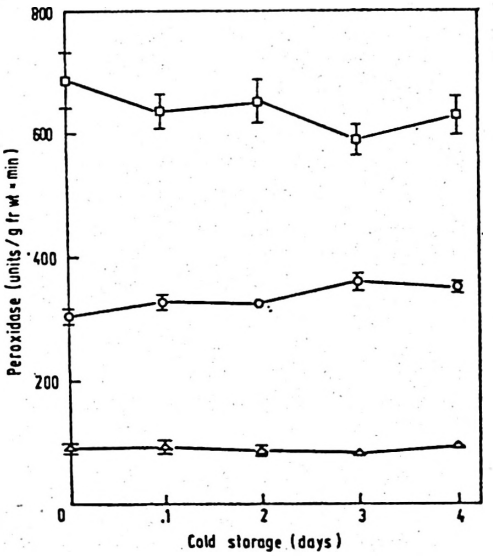

Fig. 2. *Effect of storage of leaves, stems and roots of pea plants at chilling temperature on peroxidase activity.*
Leaves (O), stems (△) and roots (□) from *Pisum sativum* L., prepared as described in the text, were stored at 0° C. Peroxidase was determined as indicated in Experimental. Each point with vertical bars represents mean ± SEM of six samples. Bars are omitted if the SEM is less than the size of the point.

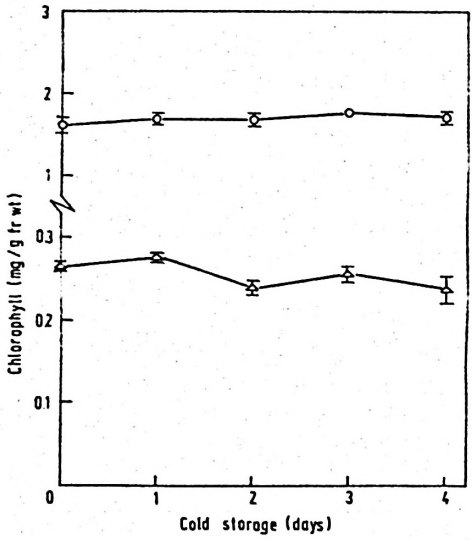

Fig. 3. *Chlorophyll content of leaves and stems from pea plants during storage at chilling temperatures.*
Leaves (○) and stems (△) were stored at 0° C during different times and chlorophyll was determined as described in the text. Each point with vertical bars represents mean ± SEM of six samples. Bars are omitted if the SEM is less than the size of the point.

As far as the root is concerned, the behaviour of catalase during cold storage is different from that of leaf ad stem (fig. 1). There was a gradual decrease in activity after the initiation of storage lasting until the third day when catalase stabilized. The depression in enzymatic activity after 1, 2, 3 and 4 days of storage was statistically significant at the 1 % level as compared with the root catalase activity at the zero time.

The peroxidase activity in leaves, stems and roots (fig. 2) followed a more constant pattern than that of catalase and did not show significant alterations during the four days of cold treatment.

Peroxisomes are the major if not exclusive sites of catalase activity in plant tissues (15). Peroxidase, contrary to previous reports, was found by PARISH (17) not to be associated with peroxisomes; it was localized in the cytoplasm and bound to cell walls, plasma membranes, mitochondria, ribosomes and nuclei (9). In our experiments, catalase and peroxidase activities of leaves and stems did not show significant variations during the four days of chilling treatment which agrees with the finding by JACOBI *et al.* (6) that the activity of enzymes considered to be organelle-related remained rather constant during the course of dark starvation in spinach as compared with that of cytoplasmic enzymes. On the contrary, in roots, where peroxisomes occur at least in small number (16), catalase was significantly depressed after storage at chilling temperatures whereas peroxidase remained unaltered. This could suggest that in root tissues stored at low temperature, the stability of the peroxisomes is lower than that of leaf and stem tissues,

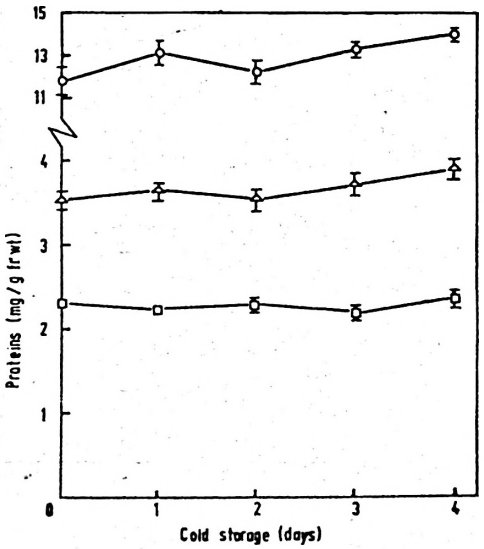

Fig. 4. *Soluble proteins of leaves, stems and roots of pea plants during storage at low temperatures.*
Leaves (○), stems (△) and roots (□) were stored at 0° C during different times. Soluble proteins were determined as described in the text. Each point with vertical bars represents mean ± SEM of six samples. Bars are omitted if the SEM is less than the size of the point.

and in these conditions, the action of proteolytic enzymes on soluble catalase could account for the decrease observed in root catalase activity during cold storage.

The chlorophyll content of leaves and stems was not significantly modified during the time of cold storage, as shown in figure 3. This result contrasts with that obtained for detached leaves of tobacco (2) kept at 25° C, where chlorophyll in the leaf dropped to a very low value within 1 to 3 days after excision. The constant levels of chlorophyll in leaves and stems here reported show that the chloroplasts in *Pisum sativum* L. are resistant against cell degradation during the treatment at 0° C.

The evolution in the content of soluble proteins during storage of the three organs of the plant is shown in figure 4. In leaves, stems and roots, soluble proteins remained nearly constant and there were no significant differences between their values after 4 days storage.

Catalase and peroxidase activities varied according to the plant organ. Leaves had the highest catalase activity, followed by stems and roots where it was 6- and 9-times lower, respectively, than that of the leaf. As for peroxidase, activity was maximum in the root while in the leaf and stem was half and 7-times lower, respectively. Chlorophyll content of leaves was 7-fold that of stems. Soluble proteins in stems and roots were 4- and 6-times lower, respectively, than those in the leaf.

The foregoing results support the conclusion that as far as total catalase and peroxidase activities, soluble proteins and chlorophyll is concerned, leaves and stems of *Pisum sativum* L. can be stored at 0° C for at least 4 days without statistically significant variations be appreciated. On the contrary, in roots under the same conditions catalase is strongly depressed whereas the other parameters studied remain nearly unaltered.

Acknowledgements

The authors thank Miss M. Garrido-Ortiz and Mr. A. Melgar-Porcel for skilled technical assistance, and Dr. A. Leal-López for helpful discussions.

Resumen

Se han estudiado los efectos de bajas temperaturas sobre las actividades catalasa, peroxidasa y contenidos en clorofila y proteínas de hojas, tallos y raíces separadas de plantas de *Pisum sativum*.

En tejidos de hojas y tallos, el almacenamiento a 0° C no produjo variaciones significativas en los niveles de los enzimas hemínicos ni en los contenidos en clorofila y proteínas, a lo largo de cuatro días. En la raíz, por el contrario, la actividad catalasa experimentó un descenso estadísticamente significativo durante todo el tratamiento, mientras que los demás parámetros permanecían casi constantes.

Los resultados obtenidos muestran la utilidad que representa la conservación del material vegetal a 0° C para la estabilización de la catalasa, peroxidasa y clorofila de hojas y tallos, así como de la actividad peroxidásica de las raíces.

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