Peroxidase Activity in Onion Bulbs of Long and Short Dormancy[1]

B. W. Poovaiah[2], Grant Vest[3] and H. P. Rasmussen[3]
Michigan State University, East Lansing

Abstract. **The peroxidase activity in bulbs of 3 onion (*Allium cepa* L.) genotypes known to differ in their dormancy characteristics was investigated at 2 stages of bulb development. Peroxidase activity was estimated by the oxidation of p-phenylenediamine, and peroxidase isozymes were studied by acrylamide gel electrophoresis. The peroxidase activity of all 3 genotypes was the same in young bulbs (1.5 – 2 cm, diam), but in mature bulbs peroxidase activity differed with genotype. Bulbs with short dormancy had higher activity, and a greater no. of isozymes than bulbs with long dormancy. A commercial preharvest spray of maleic hydrazide (MH) did not affect peroxidase activity in bulbs of either dormancy type.**

The ability of onion to remain dormant (not sprout) is a desirable storage characteristic thus, long-dormant germplasm is being incorporated into some cultivars. Currently, the sprouting of onion bulbs can be delayed by spraying onion plants with MH prior to harvest (6, 11).

Although length of dormancy is genetically controlled, environmental factors can also affect its duration. Bulbs stored at high temp have a higher percent of sprouting than bulbs stored at lower temp, and injured bulbs sprout before non-injured bulbs (1, 3).

There are reports (7, 9) that auxin levels increase in onion bulbs prior to sprout elongation and growth. Because of the association of peroxidase with some auxins this study was done to ascertain the peroxidase activity in onion genotypes known to differ in length of dormancy, and to evaluate the possibility of using any existing relationship between dormancy and peroxidase activity as a means of screening for bulbs with long-dormant genotypes.

The onion genotypes studied were 'Ruby' (red), 'Southport White Globe' (white), and 'MSU (2935 x 2879)4535' (yellow). In commercial storage (36-38F) 'Ruby' and 'Southport White Globe' have shorter dormant periods than 'MSU (2935 x 2879)4535'. Bulbs of each genotype were analyzed at 2 stages of development: a) as young developing bulbs (1.5 – 2 cm diam), and b) as mature bulbs stored at approx 2.2°C for 2 months after being cured. The young bulbs were frozen immediately after being harvested. The mature bulbs were harvested the 1st week of Sept., cured for 3 weeks, then stored until the last of Nov. The majority of these bulbs were frozen until analyzed. However, some mature bulbs of each genotype were kept at room temp and tested periodically for peroxidase activity until the end of Feb. In addition, comparisons of peroxidase activity were made between bulbs of 'Ruby' and 'MSU (2935 x 2879)4535' sprayed with MH and bulbs of these genotypes that had not been sprayed.

Each replication of each analysis consisted of using a total of 2 g of fresh tissue (parts of the stem and main axis) from the base of 5 bulbs. The tissue was ground in a mortar with a pestle in 5 ml of 0.1 M Hepes buffer (pH 7.3-7.4) with 0.1 mM dithiothreitol, and 1.0 g insoluable PVP added. The extract was strained through acetate cloth, and centrifuged at 20,000 x G for 30 min (5).

Peroxidase activities were estimated by measuring (at 485 mμ) the oxidation of 0.05 M p-phenylenediamine by 0.1 ml of the supernatant (10). Controls included H_2O_2 deficient medium and heat-inactivated extract. Estimates of peroxidase activity were determined from multiple measurements in each of 5 replications.

Enzyme extract (0.1 ml) containing about 500 μg of protein (4) was applied to each gel and electrophoresis, following the method of Davis (2), was conducted at 2 ma/tube until the marker dye neared the bottom of the gel (approx 3 hr). Following separation of the peroxidase isozymes the gels were stained for 20-30 min in a benzidine reaction mixture (10).

There was no difference in peroxidase activity in the young bulbs of the 3 genotypes. However, there was an increase in peroxidase activity, and differences between genotypes, in the mature bulbs (Fig. 1). The greatest increase and highest peroxidase activity occurred in 'Ruby', the onion with the shortest dormancy. The long-dormant onions 'MSU (2935 x 2879)4535' showed the least change and lowest peroxidase activity. The changes in 'Southport White Globe' were intermediate.

The peroxidase activity of 'Ruby' and 'MSU (2935 x 2879)4535' bulbs treated with a preharvest spray of MH was not measurably different from the peroxidase activity of unsprayed bulbs.

The peroxidase isozymes of the 3 genotypes were similar in the young bulbs, but were different in the mature bulbs (Fig. 2). The most noticeable change within a genotype was the apparent increase in the no. of isozymes in the red bulbs.

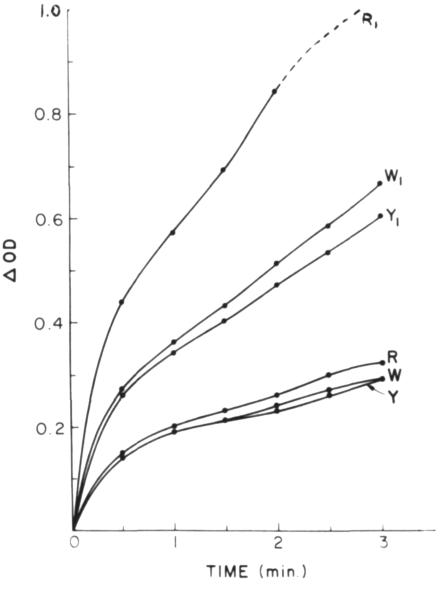

Fig. 1. Estimated peroxidase activity based on oxidation of p-phenylenediamine measured spectrophotometrically at 485 mμ. R, W, and Y represent red, white and yellow onion bulbs tested when 1.5 – 2 cm in diam. R_1, W_1, and Y_1, represent mature red, white and yellow onion bulbs.

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[2]Research Associate. Present address: Department of Horticulture, Purdue University, Lafayette, Indiana.

[3]Associate Professors, Department of Horticulture.

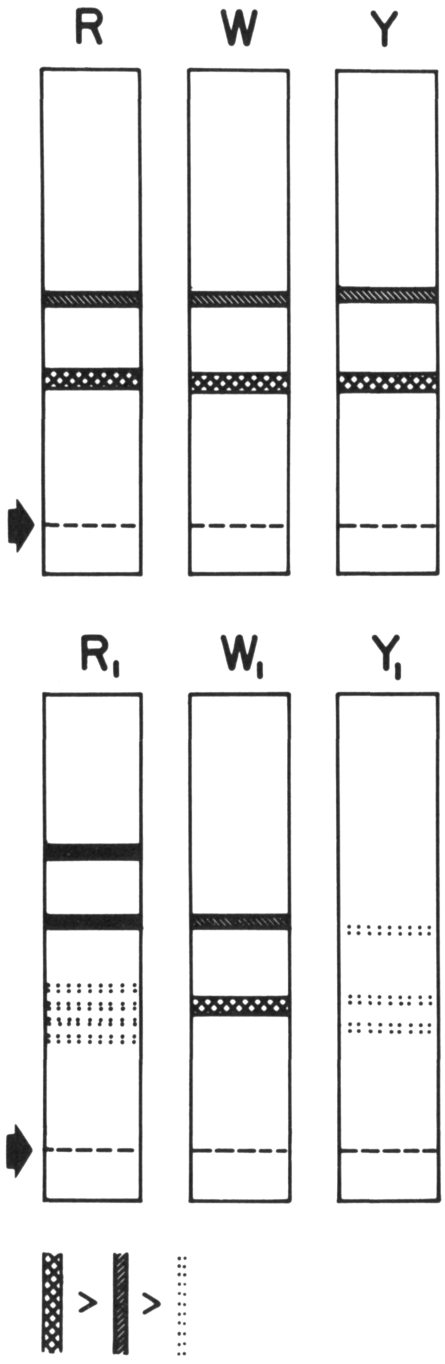

Fig. 2. Peroxidase zymograms of onion bulbs. R, W, and Y represent red, white and yellow onions tested when bulbs were 1.5 – 2.0 cm. R_1, W_1, and Y_1, represent mature red, white and yellow bulbs. The arrow indicates the marker dye.

In the bulbs kept at room temp and tested periodically there was an increase in no. and intensity of isozymes in peroxidase activity in all genotypes throughout the testing period. By this time most of the red and white onions had externally visible sprouts.

The results show that peroxidase activity was higher in stored mature onion bulbs than in newly developing bulbs, and that the 3 onion genotypes differed in their peroxidase activity. Peroxidase activity ranked 'Ruby' > 'Southport White Globe' > 'MSU (2935 x 2879)4535'. When held in commercial storage, without having been sprayed with MH, 'Ruby' will sprout before 'Southport White Globe' which sprouts before 'MSU (2935 x 2879)4535'. Since peroxidase activity is correlated in this manner with dormancy, the possibility exists that there is a relationship between dormancy or the breaking of dormancy and peroxidase activity.

Since MH inhibits sprouting of onion bulbs, but had no effect on peroxidase activity in our study, the possibilities exist that peroxidase may be associated with a different physiological stage of dormancy than MH, that there may be more than a single mechanism involved in the control of dormancy, or that peroxidase is not directly involved with dormancy or the breaking of dormancy.

Thomas and Isenberg (8) have reported that an interaction of natural auxins and inhibitor appears to control sprout activation in dormant onion bulbs. It could be that peroxidase is associated with the auxins at this stage of dormancy.

Perhaps peroxidase activity, as measured, was associated with breaking of dormancy and sprout activation. If the bulbs we used were tested after rest but before sprout elongation, MH would not have an effect on peroxidase activity. These bulbs which had the highest peroxidase activity would be the bulbs in which breaking of dormancy or activation of sprouting was most complete. In this study this would mean that the red bulbs would sprout before the others.

An internal examination of the bulbs when they were taken from storage did not reveal any observable sprout elongation or coloration. Neither were there observable internal differences between MH-treated and untreated bulbs.

It is also possible that peroxidase activity is associated with certain physiological processes which are directly involved with the breaking of dormancy, and that we were measuring an indirect association, and not a cause and effect relationship of peroxidase activity with dormancy.

The increases in peroxidase activity and number and intensity of isozymes in bulbs kept at room temp from the time of removal from storage through sprout elongation and chlorophyll development indicates an association of peroxidase with growth. This makes it imperative that the stage of bulb dormancy be carefully considered in testing for an association of peroxidase and dormancy. More research on the relationship of peroxidase activity and dormancy will need to be done before we will know if this technique will be useful in selecting for onions with long dormancy.

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