

Fig. 1. Germination of high and low vigor lima beans stored in three relative humidities at 21C.

their original viability under the latter conditions for 3 months, but beyond this period the quality of the seeds would be questionable.

These results show that low vigor lima beans can be safely stored for at least 3 years at 21C and 50% RH even though deterioration has progressed to a point where germination had dropped 17% following harvest 2 years earlier. The results also show that seedling performance of low vigor lima beans is reflected in their poor responses to poor storage conditions.

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Physiological Differences Between a Green and a Tan Dry Podded Line of Snap Bean¹

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Abstract. A green and tan dry podded line of snap bean was compared with respect to respiration rate, chlorophyll degradation, % dry matter of the leaves, effects of light and Ethrel treatment on abscission of petiole explants, and fresh weight increase of leaf discs floated on a nutrient solution. The green dry podded line respired at a higher rate, degraded chlorophyll at a slower rate and

contained a higher percentage of dry matter than the tan dry podded line. Petiole explants from the green dry podded line abscised faster in the dark and slower in the light than petiole explants from the tan dry podded line. Ethrel treatment of petiole explants resulted in promotion of abscission in both phenotypes but did not change the relative rates of abscission. Fresh weight increase in leaf discs floated in nutrient solution was greater than for the green dry podded line than the tan. These observations suggest that the green dry podded line has a higher endogenous level of kinetin or kinetin-like substance.

noted that the action of the genes resulted in the green color persisting in the leaves as well as in the pods. Leaves of plants bearing green dry pod remained attached to the plant when they senesce, while the leaves of plants bearing tan dry pod turn yellow and fall abscise. Plants bearing green or tan dry pods will be referred as green and tan plants respectively. The objective of this study was to determine if the physiological action of the genes could be inferred from comparison of the phenotypes with respect to the following physiological parameters: rate of respiration, rate of chlorophyll degradation and % of dry matter in the

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leaves; abscission of petiole explants; and fresh weight increase of leaf discs floated on a nutrient solution in the dark.

In order to investigate the effect of time after leaf removal on chlorophyll degradation, respiration rate, and % dry matter fully expanded, mature, trifoliate leaves of both phenotypes were picked and washed for 2 hr in running tap water and treated as follows: each trifoliate leaf was divided into leaflets by cutting the petiole at the base of each leaflet. One of the leaflets was analyzed immediately; the others were placed individually in 9 cm petri dishes with filter paper wetted with sterile distilled water. The petri dishes were sealed and placed in the dark at 25 C. One leaflet was analyzed after 5 days and the other after 10. Eight 1 cm discs were cut from each leaflet with a cork borer, taking care to avoid the midrib or other large veins. Four discs were washed in distilled water and placed in Warburg flasks to determine respiration rates. The remaining discs were ground in a hand homogenizer with 80% (v/v) acetone in distilled water, centrifuged, washed and the absorption measured at 645, 652, and 663 μ m. The formulae of MacKinney (5) were used to calculate chlorophyll A and B and total chlorophyll. Fresh weights of each 4 disc lot were recorded and the lots from the Warburg flasks were oven dried and weighed to determine % dry matter.

Comparison of the absorption spectra of extracts of chlorophyll pigments indicated that the ratio of chlorophyll A to B was similar in both phenotypes and that both forms of chlorophyll were being degraded in the tan plants. Further results are shown in Figures 1, 2, and 3.

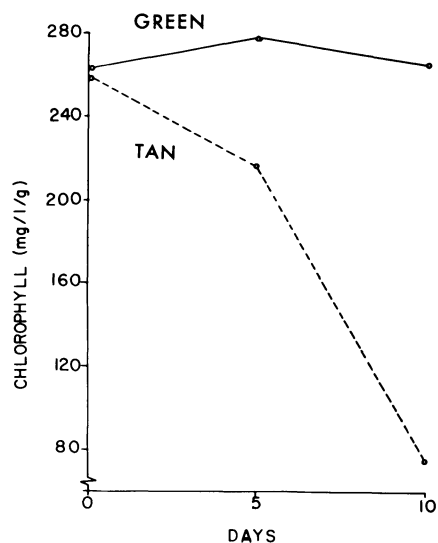


Fig. 1. Changes in concentration of chlorophyll A + B expressed as mg/l/g dry weight of green and tan phenotypes with time.

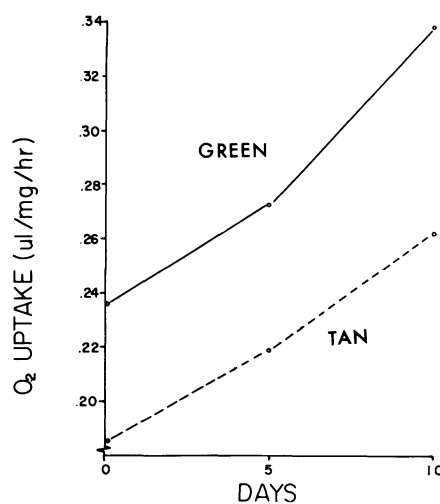


Fig. 2. Changes in respiration rate of leaf discs of green and tan phenotypes with time.

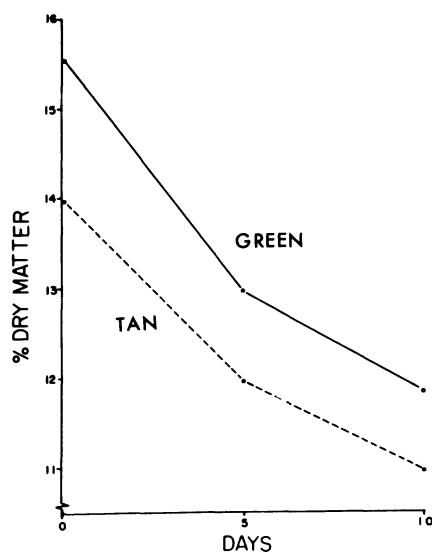


Fig. 3. Changes in % dry matter of leaf discs of green and tan phenotypes with time.

The respiration rates (Fig. 2) of the two phenotypes suggest that both are senescing even though the chlorophyll is not degraded in the green plants (Fig. 1). The higher respiration rate of the green plants may be partially explained by the higher initial percentage of dry matter and probably higher levels of available carbohydrates. Since the climateric peak is usually reached before the tissue becomes completely yellow, one may speculate that it occurred somewhere between the 5 and 10 day samples.

To study abscission, 200 petiole explants of each phenotype were prepared as follows: A 10 mm piece of petiole including 3 mm above the pulvinus was cut and washed for 1 hr in running tap water. After washing, half the explants were dipped in 100 ppm Ethrel (1) with .01% Tween⁴ for 30 sec. The explants were then placed with their proximal ends down in 2% agar in 9 cm petri dishes. Half the explants were kept in the dark, the other half were placed under cool white fluorescent light (ca. 450 ft-c).

The results of this experiment are shown in Figure 4. These data suggest that the untreated petiole explants from the green plants abscise faster in the dark than the petiole explants from untreated tan plants and slower in the light. It also appears that Ethrel treatment acts only in promoting abscission; there is no change in the relative rates of the two phenotypes.

Osborne and Moss (16) demonstrated that kinetin applied to bean petiole explants accelerated abscission in the dark. Chatterjee and Leopold (2) showed that in the light, kinetin applied to bean petiole explants retarded abscission and gibberellin accelerated abscission. It would appear therefore that the green phenotype has higher endogenous levels of kinetin or some kinetin-like substance.

Kuraishi (4) demonstrated that discs cut from radish leaves expand faster in the dark when floated on a nutrient solution containing kinetin. For the

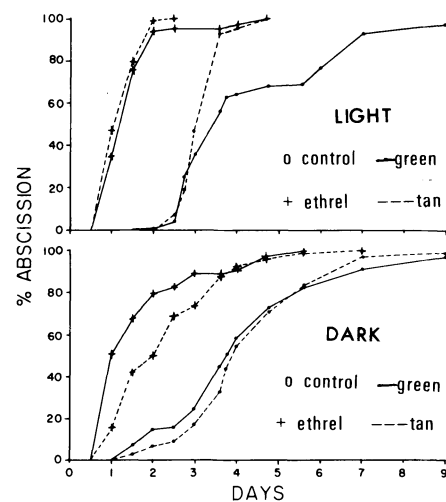


Fig. 4. Percent of petiole explants abscised by green and tan phenotypes with time.

⁴Fisher Scientific Company, Fairlawn, N. J.

study on fresh weight changes, fully expanded mature leaves were harvested from both phenotypes and kept in the dark for 24 hr. One cm discs were than cut from the leaves with a cork borer and these discs were washed for 1 hr in running tap water, blotted dry and weighed. The discs were placed in 6 cm petri dishes containing Kuraishi's solution without kinetin and kept in the dark at 22 C. Samples were removed and weighed after 8, 16 and 24 hr. The results of this experiment are presented in Figure 5. It appears that the leaves from green plants showed a greater increase in fresh weight than the tan plants. This data would tend to substantiate the hypothesis that the green plants contains higher endogenous levels of some kinetin-like substance.

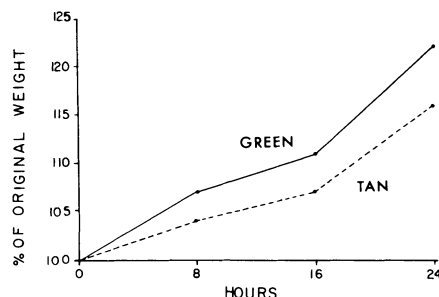


Fig. 5. Fresh weight increase of leaf discs of green and tan phenotypes with time.

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Differentiating Diploid from Tetraploid Seedlings of *Asparagus officinalis* L.¹

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Abstract. Tetraploid asparagus seedlings were produced from diploid seeds by means of colchicine treatment. Stomata length and cladophyll width were reliable characters for identifying diploids and tetraploids, but number of cladophylls per whorl and cladophyll length were unreliable.

Efficient identification of tetraploid seedlings is a problem in asparagus. Braak and Zeilinga (1) claimed to distinguish tetraploid from diploid asparagus by the longer cladophylls and darker green color of the former, but did not present supporting evidence. The present paper reports, primarily, an attempt to determine characters in asparagus (stomata length, number of cladophylls per whorl, or length and width of cladophylls) that would conveniently and reliably distinguish diploids from tetraploids.

Diploid progenies of 5 crosses were used. The method of colchicine treatment was a slight modification of the one described by Braak and Zeilinga (1). Seeds were sterilized by soaking in a concentrated solution of sodium hypochlorite for one minute, washed with distilled water and then incubated in petri dishes on moist filter paper at 30 C for 4 to 7 days until radicles emerged. When radicles were 4 to 6 mm, seed were put in vials containing a 1.5% aqueous solution of colchicine which was placed inside a desiccator under vacuum for 20 minutes. The seeds were soaked for an additional 10 minutes

under normal atmospheric pressure and then washed before sowing in peat pots.

Chromosome counts were made from root tips, pollen mother cells, and apical buds from young shoots. A hot-water bath was useful in stimulating root development of some seedlings.

In each case, the specimens were fixed in Carnoy's fixative, modification III (2), between the hours of 10 AM and 2 PM to insure the presence of numerous dividing cells. The staining procedure was adapted from Snow's squash preparation (4) using alcoholic hydrochloric acid-carmines as stain. After 24-hour fixation, the materials were washed with 3 changes of 70% alcohol, allowing at least one hour for each change. The tissues were drained in bibulous paper and then soaked in a specially prepared solution of aceto-carmines for 36 to 48 hours. The specimens were rinsed with 70% alcohol and squashed between the slide and cover slip. Only plants with 80% or more tetraploid cells in tissues examined were included as tetraploids. Varying numbers of tetraploids from the different crosses and treatment dates were obtained.

Samples of cladophylls for stomata measurement were gathered from the tenth whorl from the tip of one of the main stalks. Four or five cladophylls from each plant were fixed, using a combination of methods suggested by Clarke (3) and by Speckmann *et al.* (5). They were placed in vials with 70% alcohol and put in a bath of boiling water until most of the chlorophyll was extracted, transferred to vials containing 85% lactic acid and boiled for

approximately 10 minutes, and finally stored in lactic acid for microscopic examination.

The guard cell lengths from 5 stomata of each of the 2 cladophylls from each plant were measured in sub-units of 2.5 μ . Cladophylls were counted from 10 successive whorls of the central portion of the main shoot of each plant. One randomly selected cladophyll from each of the 10 whorls was measured for length in millimeters and for width in microns. The data analyzed were composed of means of 10 measurements.

Stomata length and cladophyll width of colchicine induced tetraploids were considerably larger than untreated diploids (Table 1). There was no overlap in ranges of diploids and tetraploids in stomata length and cladophyll width among individual plants. The data are means of five progenies, and there was no progeny x ploidy interaction; thus the results were not presented by progenies.

Chromosome counts were made from root tips from each plant but not from apical buds from all plants or from pollen mother cells of all male plants (not all of the plants had flowered). However, apical buds were studied from enough plants to indicate complete agreement with root tip counts, and there was complete agreement between root tip ploidy and stomata length and cladophyll width. We believe this is good evidence that no chimeras were involved.

The suitability of using stomata or cladophyll width or both in differentiating asparagus tetraploids

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