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Postvernalization Seed Treatment with Vitamins in Vigna catjang

Abstract. Thermoinduction for 1 week, followed by 48 hours of vitamin treatment of seeds, resulted in complete annihilation of the cold effect, the plants from the treated seeds having shown a significant increase in elongation of the root and shoot and an increase in the fresh weight as well as in the dry matter over vernalized and unvernalized controls. An increase in vegetative vigor was also noted after treatment with vitamins at lower concentrations in the unvernalized series. Cold treatment not combined with vitamin treatment resulted in a decrease in vegetative vigor.

Comparatively little work has been done on the effects on the growth of plants of low-temperature vernalization and subsequent vitamin treatment of the seeds, as compared with the tremendous amount of work that has been done on the interacting effects of low temperature and growth regulators (*1*) and on the effect of vitamins alone on the growth of plants (*2*). The experiment reported here was carried out in order to study the physiological reactions of plants from seeds subjected to low temperature and subsequent vitamin treatment. Preliminary work, carried out with the vitamin riboflavin, on the growth of *Vigna* (*3*) and pea (*4*) had shown interesting but not very significant stimulating effects on the early growth of unvernalized seeds.

Seeds of homozygous pure strains of the common Indian pulse *Vigna catjang* var. pushaphalguni, obtained from the State Agricultural Experiment Station of Orissa at Sambalpur, were used in this investigation. The seeds were soaked in distilled water for 24 hours, then subjected to low-temperature treatment at 3° to 5°C for 1 week.

The vernalized seeds were then separated into ten lots of 15 seeds each and treated with riboflavin and ascorbic acid, alone and in combination, at concentrations ranging between $10^{-3}M$ and $10^{-5}M$, for 48 hours. The seeds were then washed in distilled water, sown in earthenware pots containing garden soil, and grown under field conditions. The plants were harvested after 2 weeks, and data were recorded. In another series the low-temperature treatment was omitted; we refer to this series hereafter as the "unvernalized series." Seeds treated with distilled water and low temperature for 1 week, then treated with water for another 48 hours, served as controls for the vernalized series; the low temperature treatment was omitted for the controls for the unvernalized series. In Table 1 the data obtained for the two series are expressed as percentages of growth of the respective controls.

In the vernalized series the elongation of the root and shoot was significantly higher in the plants grown from vitamin-treated seeds than in the controls when the riboflavin and ascorbic acid were applied singly or in combination at the lower concentrations, but riboflavin alone at a concentration of $10^{-3}M$ was inhibitory. The most interesting result was the failure of the seeds to grow when the vitamins were used in combination at the highest dosage. For the unvernalized series low concentrations of the two vitamins, alone or in combination, stimulated growth, the effect being less pronounced than in the vernalized series.

The changes in the levels of weight accumulation of roots and shoots for the vernalized series were very significant. Treatment with vitamins produced a significantly progressive increase in the increment of both fresh weight and dry weight of root and shoot, with decrease in the concentrations of the vitamins used, alone or in combination. In the unvernalized series, vitamins at the two lower concentrations promoted growth if used singly and did so even more if used in combination. Calculation, from the actual dry weight of plants, of the top-to-root ratio showed that the ratio increased progressively with decrease in concentrations of ascorbic acid in the vernalized series. Riboflavin had no effect on the ratio. When the vitamins were used in combination at the two lower concentrations, the ratio was higher than it was in the controls, indicating a promotive effect on shoot growth. The ratio was not significantly altered for the unvernalized series by the vitamin treatment.

When the dry weight was expressed as a percentage of the fresh weight, it was revealed that the use of riboflavin at lower concentrations, alone or in combination with ascorbic acid, gave a higher value for root growth, indicative of a lower water content, in the vernalized series, and that the use of ascorbic acid, alone or in combination with riboflavin, at the lowest concentration gave a higher value for the shoots. No interesting results were obtained in the unvernalized series.

The investigation offers an opportunity to study the effects of ascorbic

Table 1. Data for the unvernalized (UV) and vernalized (V) series, expressed as percentages of growth of the respective controls.

Molar concentration	Length				Fresh weight				Dry weight			
	Root		Shoot		Root		Shoot		Root		Shoot	
	UV	V	UV	V	UV	V	UV	V	UV	V	UV	V
					Riboflavin							
10^{-3}	60	71	62	71	16	96	30	138	49	92	22	89
10^{-4}	99	113	64	181	118	166	120	376	110	219	113	291
10^{-5}	106	144	106	218	125	250	128	330	166	333	101	275
					Ascorbic acid							
10^{-3}	100	120	95	158	76	188	84	218	102	124	82	179
10^{-4}	108	120	128	188	131	187	143	334	164	170	126	231
10^{-5}	113	122	127	195	128	206	112	438	173	250	103	250
					Riboflavin plus ascorbic acid							
10^{-3}	38		35		51		5		5		5	
10^{-4}	83	120	99	170	128	70	110	258	164	128	125	215
10^{-5}	103	173	107	200	128	110	144	182	193	176	141	211

acid and riboflavin, used alone or in combination, and the effect of low-temperature treatment, prior to application of the vitamin or vitamins, on early growth and accumulation of dry matter in plants. It is concluded that ascorbic acid or riboflavin, or both, at low concentrations are definitely more growth-promoting for the vernalized than for the unvernalized seeds (*5*).

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Interactions of Pectin and Protein in the Heat Coagulation of Proteins

Abstract. Pectin decreases the heat coagulation of many proteins, including the soluble proteins of pea stems, ovalbumin, and bovine serum albumin. This observation probably explains the decrease in heat coagulation of proteins from pea stems following in vivo auxin administration, since auxin causes a great increase in the soluble pectin content of treated cells.

We have reported previously (*1*) that the administration of 2,4-dichlorophenoxyacetic acid or other auxins to growing pea stem tissue results in a decreased heat coagulability of the soluble proteins of the particle-free homogenate of the stem. We now believe this effect to be due to an increase induced by auxin administration in the level of cold water soluble pectins (*2*) which then interact with the proteins during heat coagulation.

Subapical sections from the stems of green or etiolated peas were harvested, homogenized in 0.001*M* EDTA (ethylenediamine tetraacetate) plus 0.5*M* sucrose at *p*H 6.0, and centrifuged as previously described (*1*). The clear particle-free supernatant fraction was used for the heat coagulation experiments and also for assays of the pectin which is soluble in cold water. Pectin was determined by a slight modification of the method of McComb and McCready (*3*), in which pectin is precipitated by the action of 70 or 95 percent ethanol for 72 hours at 2°C, demethylated in 0.05*N* NaOH for 30 minutes, and hydrolyzed by heating for 10 minutes with concentrated H_2SO_4 to the free galacturonic acid, which was then determined colorimetrically with carbazole. Because of the lack of specificity, this method was checked several times against a hydrolytic procedure involving purified polygalacturonase (*4*). Furthermore, the in vivo synthesis of the material assayed by the above procedures was inhibited by $10^{-3}M$ ethionine, and this inhibition was reversed by methionine. Both facts give strong support to the view that the material assayed was pectin.

Table 1 shows the increase in the cold water soluble pectin content of green pea stem sections which had been incubated for 20 hours in the light in $10^{-5}M$ 2,4-dichlorophenoxyacetic acid (2,4-D), 1 percent sucrose, and 0.01*M* phosphate buffer, *p*H 6.1. It is clear that the growth increase induced by 2,4-D is accompanied by a massive synthesis of pectins, as well as by the decreased heat coagulability of proteins previously reported (*1*).

We next investigated the possibility that the deliberate addition of pectin in vitro could accomplish the same effects on protein coagulation as did the in vivo administration of 2,4-D. To the clear particle-free homogenate of pea stem were added varying quantities of commercial citrus pectin. Aliquots (5 ml) in test tubes were immersed in a boiling water bath for 10 minutes. The heat coagulum was centrifuged at 3000*g*, washed twice with cold distilled water, poured into a previously tared aluminum weighing dish and dried overnight at 90°C. Table 2 shows the relation between pectin concentration and the dry weight of the precipitate so obtained. This effect of pectins in reducing the heat coagulability of pea proteins could be observed only over the rather narrow range *p*H 5.5 to 6.5, as shown in Table 3. The inhibitory effect of the pectins disappears below *p*H 5.0, where ionization of pectin carboxyls is repressed, and above *p*H 7.0, where pea proteins fail to coagulate by heat.

Similar experiments were undertaken with various commercially available proteins, including bovine serum albumin, and with freshly prepared ovalbumin. Typical results are shown in Fig. 1. While these curves differ in detail, they both show that pectins inhibit heat coagulation in the general range *p*H 5.3 to 5.7, and promote heat

Table 1. Effect of the auxin, 2,4–dichlorophenoxyacetic acid, on growth and the content of cold water soluble pectin in sections of green pea stems.

2,4–D (mole/liter)	Increase in fresh wt. (%)	Uronic acid equiv. (μg/g)	
		In original fresh wt.	In final fresh wt.
0	46	349	239
10^{-5}	124	870	382

Table 2. Effect of commercial citrus pectin on heat coagulation of pea stem proteins at *p*H 6.0

Pectin concn. (μg/ml)	Coagulum (mg dry wt.)
0	4.9
6.6	4.6
13.3	4.5
20.0	3.4
26.6	1.4
100.0	0.2

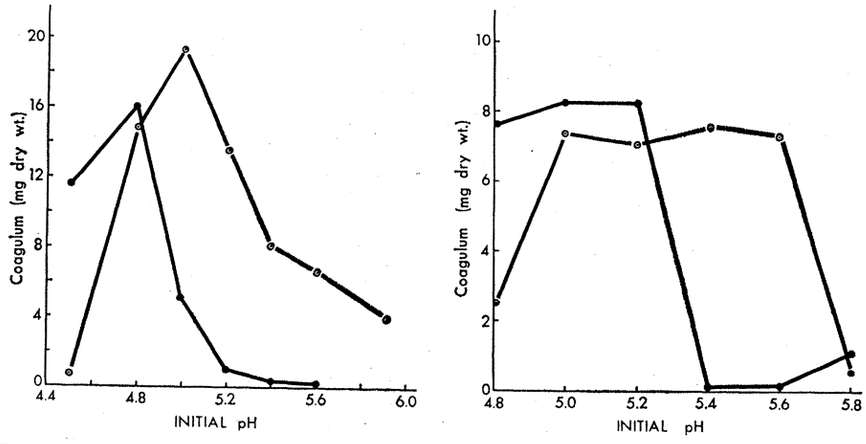

Fig. 1. Effect of *p*H on the heat coagulability of bovine serum albumin (left) and ovalbumin (right) in the absence (open circles) and presence (solid circles) of commercial citrus pectin (100 μg ml).