Learning of plant adaptations to environmental stresses: experiments with proline

Aprendizaje sobre las adaptaciones de las plantas a las tensiones ambientales: experimentos con el proline

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Abstract

Adverse environmental conditions induce the accumulation of 'stress metabolites' in plants. Of these metabolites proline is probably the most widespread. It has been shown that proline accumulates under conditions of water shortage, high salinity, chilling, heat, heavy metal exposure and ultraviolet stress. Some possible roles for proline in plants are reviewed. A bioassay using plants is suggested, as well as a range of possible experiments suitable for biology students.

Key words*: interactive learning, plant adaptation, environmental stress, proline.*

Resumen

Las condiciones ambientales adversas inducen a la acumulación de los metabolitos de la tensión en plantas. De estos metabolitos el proline, es probablemente el más conocido. Se ha demostrado que el proline se acumula

bajo condiciones de escasez de agua, de la alta salinidad, de enfriarse, del calor, y de la exposición del metal pesado y de rayos ultravioletas. Algunas influencias posibles del proline en plantas se analizan. Se sugiere una prueba biológica con plantas, así como varios posibles experimentos convenientes para los estudiantes de biología.

***Palabras clave**: aprendizaje interactivo, adaptación de plantas, tensión ambiental, proline.*

INTRODUCTION

Plant physiology experiments have become less popular in many schools at university levels, not only as a result of the misconception that such activities present a significant source of danger to the health and safety of pupils but also because of the cost in terms of time and consumables. In fact, there are a wide range of physiology *experiments* that can be carried out with a minimum of effort and equipment. Organizations such as the National Centre for Biotechnology Education (NCBE) and Science and Plants for Schools (SAPS) have produced useful protocols, and a recommended list of microbes suitable for schools is available.

The overwhelming impression (Rabe, 1990), when we look at plants on a windowsill, or out in the countryside, is that plants face frequent periods of environmental stress that impairs their growth and reproductive capacity. Environmental parameters with deleterious effects include unfavorable climates, water stress, and inappropriate soils (not suitable). Restrictions of plant growth cannot be attributed to one single process, because plant growth is the result of many integrated and regulated physiological and biochemical processes. In order to survive and maintain minimal growth potential, plants must conform to these extreme environments *by means* of adaptive changes in metabolism and cell composition. Among the various mechanisms enabling plants to cope with water stress the most common is the accumulation of intracellular solutes such as sugars and free amino acids, which are compatible solutes. The most frequent nitrogen-containing compounds that accumulate in plants subjected to environmental stresses are amides (glutamine and asparagines), amino acids (arginine, proline, citrulline, and ornithine), and polyamines (putrescine). The accumulation of proline *upon* dehydration due to water deficit or increasing osmotic pressure is the most recent *finding which* suggests the osmoregulatory role of proline in environmentally stressed plants. Generations of students have extracted and separated amino acids, using thin-layer chromatograms sprayed with ninhydrin, but far fewer students have studied the presence of the amino acid proline. This paper suggests a range of *experiments* using proline.

PROLINE

The pathways of proline biosynthesis are known in higher plants: the glutamate pathway and the ornithine pathway. These pathways seem to be identical for all organisms. Most evidence about the roles of particular enzymes in proline biosynthesis or catabolism has been obtained from micro organisms. The ability of plants to degrade proline through an oxidation process has been shown clearly by Stewart (1981). The catabolism of proline involves the conversion to glutamic acid via pyrroline-5-carboxylate reductase and a subsequent metabolism of glutamate by the Krebs cycle reactions that release CO_2 as the end product. Oxidation of proline is catalyzed by proline oxidase and requires oxygen as an oxidant. Proline shows a conspicuous ability to control its own biosynthesis. Exogenous application to plant tissues of an amount of proline sufficient to increase the endogenous pools enhanced the rate of proline oxidation as a result of the feedback inhibition process (Boggess *et al*., 1976; Stewart, 1972). It is known that proline oxidase, one of the enzymes involved in proline degradation, can be induced by high concentrations of proline (Adams and Frank, 1980; Boggess *et al*., 1976). Feedback inhibition of proline synthesis does not occur under water-stress conditions.

BIOLOGICAL ROLES OF PROLINE

It has been shown that proline accumulates under conditions of water shortage, high salinity, chilling, heat, heavy metal exposure and ultraviolet stress (Atici, *et al*., 2003; Demir, 2000; Demir and Kocaçaliskan, 2002). It plays a major role in osmoregulation and osmotolerance (Wyn-Jones and Storey, 1978). According to Stewart and Lee (1974), proline is a substance inducing osmotic adjustment. Moreover, it has been shown to protect enzymes from inactivation by salinity, heat, chilling and dilution *in vitro* (Aziz *et al*., 1997). For example, proline activated catalase, peroxidase and polyphenol oxidase enzyme activities *are thus protected* in spinach (Öztürk and Demir, 2002). The ability of proline to activate the enzymes may suggest a limited conformational change. The accumulation of proline in a wide variety of both halophytes and non-halophytes when subjected to various stresses and the role of proline in adaptive responses has been reviewed (Flores, 1991). Proline has been assigned the role of a cytosolute, a storage compound or a protective agent for cytoplasmic enzymes and cell structure (Ahmad *et al*., 1982; Cramer *et al*., 1989), stabilizing proteins (Schobert and Tschesche, 1978). Hanson and Hitz (1982) suggested that proline accumulation is a consequence of stress-induced damage to cells. In plants, the role of proline may not be restricted to that of a compatible osmolyte. Proline accumulation may reduce stress-induced cellular acidification and proline itself may act as a substrate for respiration, which might provide energy needed for recovery from stress. In transgenic tobacco plants the enhanced activity of proline biosynthesis improves tolerance to hyperosmotic stress (Kishor *et al*., 1995). Hanson *et al* (1977) considered proline accumulation to be a symptom of damage. However, many *researchers* have ascribed a positive role to proline associated with some sort of adaptive response. Other researchers have suggested that proline is a source of energy, carbon and nitrogen for the recovery of tissues (Blum and Ebercon, 1976). However, some researchers claim that proline accumulation is a coincidental result of metabolic irregularities created by salt stress, and therefore it has no adaptive value (Fukutoku and Yamada, 1984; Tipirdamaz and Çakirlar, 1990).

METHODS

Approximately 0.5g of plant material was homogenized in 10 ml of 3% aqueous sulphosalicylic acid and the homogenate filtered through Whatman # 2 filter paper. Two ml of filtrate was *combined* with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube and *the reaction was allowed to proceed* for 1 hour at 100 °C, and then was terminated in an ice bath. The *acid-ninhydrin* was prepared by warming 1.25g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid, with agitation, until dissolved. Kept cool (stored at 4 °C) the reagent remains stable for 24 hours. The reaction mixture was extracted with 4 ml toluene, *and* mixed vigorously with a test tube stirrer for 15-20 sec. The toluene containing chromophore was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene for a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

[(Mg proline/ml X ml toluene) / 115.5 µg/mmole]/ [(g samples)/5] = µmoles proline/g of fresh weight material (Bates *et al*., 1973).

PROLINE CONTENT OF GERMINATING WHEAT GENOTYPES UNDER ULTRAVIOLET LIGHT

The effects of ultraviolet light on the proline content of four (wheat cultivars) *varieties of domesticated wheat* (*Triticum aestivum* L., cvs. Bezostoja-1, Dogu-88, Turkey-13, Yayla-305) *were* investigated. The seeds were surface sterilized with 1% sodium hypochlorite. 25 seeds were placed in 12 cm petri dishes furnished with 2 sheets of Whatman n° 1 filter paper moistened with 10 ml of distilled water, and they were left to germinate in an incubator at 25 °C under continuous ultraviolet light for seven days. Every day the proline content in the coleoptile and radicle *was* determined using the acid-ninhydrin method. Ultraviolet light increased the daily proline content of both the radicle and coleoptile (table 1) (Demir, 2000).

Table 1
Proline content (mM/g fresh weight) in the radicle and coleoptile in seedlings from germinating wheat germinated seed under ultraviolet light. Radicle (R), Coleoptile (C)

Cultivars	5th day				6th day				7th day			
	Control		UV		Control		UV		Control		UV	
	R	C	R	C	R	C	R	C	R	C	R	C
Bezostoja-1	12.4	5.7	15.4	9.4	17.9	9.2	27.0	18.2	21.6	14.3	26.3	17.7
Dogu-88	11.8	5.2	17.2	16.1	10.8	4.7	25.4	23.0	10.3	8.5	21.0	16.4
Turkey-13	16.2	8.7	14.8	9.0	22.4	10.8	24.3	18.6	14.0	7.8	34.0	31.8
Yayla-305	17.2	11.8	15.6	17.1	27.3	13.6	30.0	30.2	19.6	13.0	22.1	16.1

LSD (0.05): 2.4

EFFECTS OF LOW TEMPERATURE ON PROLINE CONTENT IN WINTER WHEAT AND CABBAGE LEAVES

The content of proline in winter wheat (*Triticum aestivum* cvs. Doğu-88) and cabbage leaves (*Brassica oleracea* cvs. Acephala*)* during acclimation to low temperature was investigated. Winter wheat (*Triticum aestivum* cvs. Dogu-88) and cabbage (*Brassica oleracea* cvs. Acephala*)* seeds were grown for 45 days as a control on vermiculite with Hoagland solution at 20/18 °C in a greenhouse with a photon flux density of 125 ìmol m^{-2} s^{-1} and

a light/dark period of 16/8 h. Cold treatments were performed by moving the seedlings into a growth chamber (Sanyo Co. Japan) (table 2) according to GRIFFITH (1993). Non-acclimated control plants were kept in the greenhouse. Samples from cold acclimated and non-acclimated plants were taken at 15 day intervals. When both of the plants species were cold acclimated, proline contents were higher than in the controls (non-acclimated) (figure 1) (ATICI *et al*., 2003).

Table 2
Growth conditions for cold acclimation

Time [d]	Temperature [°C] [day/night]	Day-length [h] [light]
1-7	20/18	16
8-13	17/15	15
14-19	14/12	14
20-25	11/9	12
26-31	8/6	10
32-37	5/2	9
38-45	5/2	8

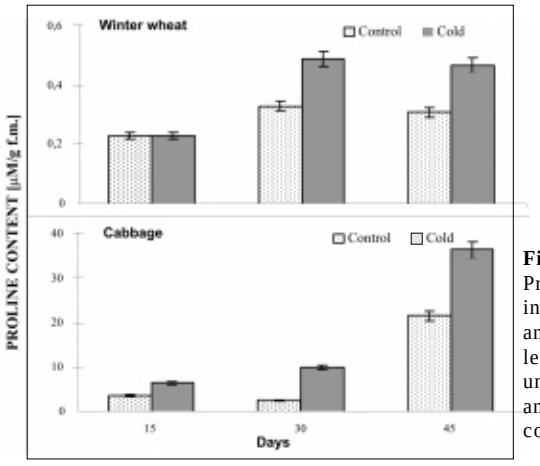

Figure 1
Proline content in winter wheat and cabbage leaves grown under control and cold conditions

DISCUSSION

Adverse environmental conditions induce the accumulation of 'stress metabolites' in plants. Of these metabolites proline is probably the most widespread. It has been shown that proline accumulates under conditions of water shortage, high salinity, chilling, heat, heavy metal exposure and ultraviolet stress. The accumulation of proline in plants subjected to environmental stresses has been widely, although not universally, observed. There are three possible causes for the accumulation of free proline under stress: first, stimulation of proline synthesis from glutamic acid, which has been found to be dependent on the abscisic acid concentration; second, inhibition of proline oxidation to other soluble compounds; and, third, inhibition of protein synthesis (DEMIR, 2000). Genotype variations are very common; however, a positive correlation cannot always be found between the proline content and a plant's relative tolerance or susceptibility. Restoring plants to optimal growth conditions results a rapid decline in the proline content (DEMIR and KOCAÇALISKAN, 2002).

As a result, the induction of proline accumulation by water shortage has been ascribed to several possible physiological functions. These functions include osmoregulation, a soluble N sink, a signal of senescence, and an indicator of plant resistance to stress. Proline may affect the solubility of various proteins, thus protecting them against denaturation under water-stressed conditions (BLUM and EBERCON, 1976, SINGH *et al*., 1973).

CONCLUSIONS

Proline accumulation is an interesting field, worthy of detailed investigation and exploration. It gives students the opportunity to learn and to apply their knowledge of basic mathematical data analysis. It is relatively simple to observe proline accumulation and environmental stress by using a spectrophotometer and, hence, is extremely suitable for use in high school biology laboratories.

If they have learnt the properties of proline from the laboratory exercise, they can understand the relationships between proline accumulation and environmental stresses in the plant. Thus this laboratory exercise could also be useful for biology classes in universities.

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