Table 1 *Amylase production by seed moulds of pearl millet in three liquid media*

Species	Activity zone (cm)		
	Glucose medium	Starch medium	Pearl millet flour medium
Alternaria alternata	1.4	2.1	1.8
Al. tenuis	1.6	2.5	2.4
Aspergillus flavus	1.7	3.5	1.9
As. niger	2.2	1.6	0.9
Cladosporium cladosporoides	0.0	0.0	0 0
Curvularia lunata	1.5	3.0	2.0
Cu. pallescens	1.4	1.6	1.7
Drechslera longistrata	1.6	1.6	1.9
D. tetramera	2.4	1.9	1.6
Fusarium moniliforme	1.4	2.3	2.0
F. oxysporum	2.0	1.9	1.5
F. scitectum	1.7	2.1	2.1
Penicillium funiculosum	1.3	2.0	2.0
Pythium sp.	2.5	2.7	2.1
Memnoniella sp.	0 0	0.0	0.0
Rhizoctonia solani	1 9	2.5	2.4
Rhizopus stolonifer	1.9	2.1	1.0

cavity (0.08 cm diameter) in the centre of the plates. After incubation at 28°C for 48 h, the plates were flooded with Lugol's iodine solution. The width of the non-blued zone around the cavity was measured and the results were recorded as the average of two replicates.

It is clear from table 1 that of the seventeen fungi tested, *A. flavus* and *C. lunata* were highly efficient in amylase production, forming 3.5-cm and 3.0-cm zones respectively. *Cladosporium cladosporoides* and *Memnoniella* sp. were not capable of amylase production.

It is interesting to note that the starch medium was superior, compared to glucose and pearl millet flour media, for amylase secretion by all the fungi except *Aspergillus niger*, *Curvularia pallescens*, *Drechslera longistrata*, *D. tetramera* and *Fusarium oxysporum*. Pearl millet flour medium was the test for *C. pallescens* and *D. longistrata*, while glucose medium was the best for *Aspergillus niger*, *D. tetramera* and *F. oxysporum*. In addition to starch, pearl millet contains proteins and lipids. The superiority of pearl millet flour medium over glucose medium can be attributed to the additional nutrients, which probably stimulated amylase secretion. Similar work was done on jowar flour medium by Wadje and Deshpande[7].

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PHYSIOLOGICAL BASIS FOR THE INHIBITION OF NITROGENASE ACTIVITY IN WATER-STRESSED COWPEA (*VIGNA UNGUICULATA* (L.) WALP)

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NITROGENASE activity was found to be extremely sensitive to water stress in many leguminous crops[1-3]. Even a mild water stress (0.5 MPa decrease in plant water potential) leads to more than 50% drop in total nitrogenase activity (TNA) in many crops[1,3,4]. However, other physiological processes like photosynthesis, transpiration and nitrate reduction are comparatively less affected at a similar plant water status[2,4]. But there has been a controversy whether the high sensitivity of nitrogenase activity is due to dehydration effects on the enzyme in the nodules or indirect causes like limitation of photosynthate supply from the shoot. In earlier studies, Huang *et al.*[5] indicated that in water-stressed soybean, photosynthate supply from the shoot essentially limited nitrogenase activity in the nodules. However, more recent studies[6-8] have indicated direct effects of dehydration on the nodules to be primarily responsible for the drop in nitrogen fixation rate in soybean and other annual legumes. In the present communication we report the results of stem girdling experiments designed to evaluate the role of host photosynthate limitation on the inhibition of nitrogenase activity in normal and water-stressed cowpea plants.

Cowpea plants (cv. C-152) were grown in glazed

pots of 15 kg capacity filled with loamy sand soil (pH 6.5, organic carbon 0.52% and total nitrogen 0.05%). The pots were kept under natural illumination and temperature in a greenhouse (1500 μmol s^{-1} m^{-2} light intensity, 30–35°C day temperatures and 14-h photoperiod) and irrigated with tap-water. The treatments were imposed when the plants were 30 days old. The pots were divided into two equal sets, and one set was subjected to water stress by withholding water three days prior to sampling, while the other set continued to receive daily watering. Each of these sets was further subdivided into two groups. Plants were girdled according to the procedure described earlier[5] in one group while the other set was left ungirdled. All observations were made 24 h after girdling.

The rates of photosynthesis and transpiration were measured in the uppermost fully expanded leaves of plants in each treatment using a LI-6000 portable photosynthesis system (Licor Inc., USA). Leaf water potential (Ψ_{leaf}) was measured using a Scholander pressure bomb (PMS Instruments Inc., USA). Nitrogenase activity was assayed by acetylene reduction assay (ARA) as described elsewhere[9]. Total soluble sugars in nodules and roots were estimated by the phenol–sulphuric acid method[10] using 200 and 500 mg fresh material respectively. Total ureide content of dried stem and nodules was determined by the phenylhydrazine method following extraction with equal volumes of ethanol and 0.1 M phosphate buffer (pH 7.2)[9].

Both water stress and girdling caused significant reduction in total (TNA) and specific (SNA) nitrogenase activity (table 1). Water stress led to a significant reduction in both fresh and dry weights of nodules per plant, while stem girdling alone resulted in only a marginal decrease in nodule fresh weight. In stressed plants, therefore, part of the reduction in TNA was due to the reduction in fresh weight of the nodules because of shedding, whereas the reduction in SNA in all the treatments is essentially due to the stress effects on the activity in the existing nodules. The significant reduction in SNA of well-watered plants within 24 h after girdling indicates that any short-term impediment in phloem translocation causes severe inhibition of nitrogenase activity in nodules. In this context it may be relevant to mention that short-term limitation of current photosynthate supply was found to restrict nitrogenase activity in soybean[5], while in perennial species like alfalfa reserve carbohydrates in the nodules supported the nitrogenase activity despite complete removal of canopy[11].

Plant water status (Ψ_{leaf}), and photosynthesis and transpiration rates did not differ significantly in stem girdling without water stress, but under water stress there was a significant decrease in all these parameters. However, soluble sugar content in roots and nodules declined significantly due to girdling (table 2), indicating a possible restriction of photosynthate flow from shoot.

In the stressed plants, no difference was found between girdled and ungirdled plants in SNA of nodules. Similarly, rates of photosynthesis and transpiration, and leaf water potential did not differ. However, stressed and girdled plants had lower total soluble sugars in roots and nodules than stressed, ungirdled ones. But stressed plants, girdled or ungirdled, had higher soluble sugars in nodules than well-watered plants. The increase in soluble sugars in water-stressed plants may be due to the hydrolysis of polysaccharides normally associated with water stress or transport of a greater proportion of photosynthate to roots and nodules at the expense of shoot, as was found in soybean[12].

Thus the data show that the direct effects of dehydration on nodules, either causing shedding or affecting the functioning of existing nodules, become overwhelming under water stress compared to all the other factors, including the supply of assimilates

Table 1 *Effect of water stress and stem girdling on photosynthesis, transpiration, nitrogenase activity and nodule biomass of cowpea plants*

	Photosynthesis (mg CO_2 m^{-2} s^{-1})	Transpiration (mg H_2O m^{-2} s^{-1})	TNA (μmoles C_2H_4/plant/h)	SNA (μmoles C_2H_4/g nodule, h)	Nodule dry weight (mg/plant)
No stress, no girdling	0.549	263	2.400	28.58	82.00
No stress, girdling	0.480	251	0.231	4.65	58.67
Stress, no girdling	0.210	167	0.025	1.09	32.00
Stress, girdling	0.260	173	0.022	0.89	30.73
± SE of mean	0.082	14	0.066	0.67	5.82

Table 2 *Total soluble sugar and ureide content of different plant parts of cowpea under water stress and girdling*

	Total soluble sugars (mg g^{-1} dry wt)		Total ureides (μmol g^{-1} dry wt)	
	Nodules	Roots	Nodules	Shoots
No stress, no girdling	68.20	36.23	5.41	41.42
No stress, girdling	45.83	31.13	6.91	18 08
Stress, no girdling	104 60	69.40	13.61	67.71
Stress, girdling	66.50	48.40	12.74	61.25
± SE of mean	6 07	6 46	1.21	2.65

from the shoot. The drastic reduction in TNA under stress therefore may not be primarily related to the availability of carbon skeletons *per se* in the nodules. The reduction in nitrogenase activity may have to do with enzymes concerned with the supply of energy and carbon skeletons to the nitrogenase reaction in nodules besides effects on nitrogenase itself. Limitation of oxygen supply to the bacteroids due to structural changes in stressed nodules was shown to be an important factor affecting nitrogenase activity in soybean[8]. We have also noticed a severe decline in nodule respiration preceded by an initial increase with developing water stress in several legumes including cowpea (unpublished data).

It is well known that many legumes, including cowpea, transport the fixed nitrogen from nodules to shoot as ureides[13]. In the present study total ureide content of nodules and stem was determined in plants harvested and dried immediately after sampling, as it has been shown that tissue ureide content in cowpea agrees with results of xylem sap analysis for relative abundance of ureides at a given time[14]. In well-watered plants girdling caused an accumulation of ureides in nodules and reduction in ureide level in shoot (table 2). One possible reason for this might be that the stem girdling resulted in deprivation of carbon skeletons needed for the export of ureides from nodules to shoot. However, in stressed plants, girdling did not make any difference in ureide levels. In fact there was a heavy accumulation of ureides in both nodules and shoot. Higher concentrations of tissue ureides in water-stressed plants have been generally observed and are attributed to the low water solubility of ureides as well as to impediments in their further metabolism in shoot[3,9]. Thus, in well-watered plants, stem girdling seems to affect the export of ureides into the shoot, and their accumulation in nodules may possibly inhibit nitrogenase activity. But in stressed plants, any differences in concentration of ureides in relation to stem girdling are not obvious owing to the overwhelming effects of dehydration.

Our results indicate that, in well-watered cowpea plants, a short-term impediment in the translocation of sugars, due to stem girdling, results in a sharp decline in nitrogenase activity by limiting the supply of current photosynthate to the nodules. Carbon skeletons for the export of fixed nitrogen from nodules to the shoot may also be affected. However, when the plants are under water stress, the direct effects of dehydration on nodules become more overwhelming and critical in inhibiting nitrogenase than the photosynthate supply from shoot.

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EFFECT OF STIK ON SPROUTING IN THE STEM CUTTINGS OF *PYRUS PASHIA* BUCH.-HAM. EX D. DON

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THE physiomorphological effect of application of various growth regulators on rooting in stem cuttings has been studied by several workers[1-7]. The growth regulators extensively used by these workers were auxins, especially indoleacetic acid (IAA) and naphthaleneacetic acid (NAA). Though all the auxins have similar qualitative effects, they elicit different responses by the plant owing to differences in their chemical properties[2]. A new growth regulator, Stik (whose active ingredient is NAA as the sodium salt), has also been used and its stimulatory effect on rooting and regeneration of stem cuttings of *Aerva sanguinolenta* has already been proved[8]. This growth regulator has also been shown to stimulate the activity of lenticel meristem in *Sapium insigne*[9].

Stem cuttings of approximately equal diameter (8–10 mm) and length (20 cm) were taken from a healthy *Pyrus pashia* tree growing at an altitude of 1500 m above MSL. Five concentrations, viz. 0.1, 0.2, 0.5, 1.0 and 2.0 mg/ml, of Stik were prepared in distilled water. The cuttings were grouped into batches of 25 for each of the five concentrations and their basal ends were dipped in Stik to a depth of 5 cm for 48 h at room temperature (13 ± 1°C). Control cuttings were dipped in distilled water. All the cuttings were then placed up-side-up in pots containing garden soil and kept under laboratory conditions (9–17°C). The upper ends of the twigs were covered with moist cotton to avoid drying. The experiment was started in the middle of December and the final observations were recorded after five weeks.

Stik has proved to be very potent in inducing sprouting in stem cuttings of *P. pashia*. Although the control set also exhibited sprouting, it was very much less than the sprouting in 0.1 and 0.2 mg/ml of Stik. Further, the adventitious buds produced on cuttings of the control set did not increase much in length. A visible sign of initiation of sprouting was observed first on cuttings treated with 0.1 mg/ml Stik on day 12 of treatment. Cuttings treated with 0.2 and 1 mg/ml Stik showed sprouting on day 15 and those treated with 0.5 and 2 mg/ml on days 17 and 19 respectively. In the control set, sprouting could be observed only on day 22. For all the parameters studied, 0.1 mg/ml was the most potent concentration, followed by 0.2 mg/ml. The leaves were of normal shape and size on cuttings treated with 0.1 and 0.2 mg/ml Stik and on control cuttings (figure 1A–C), but were variously deformed in the other cases (figure 1D–F). In the case of 1 and 2 mg/ml Stik, the leaves became narrower, longer and leathery (figure 1E, F).

The above observations indicate that Stik has a marked influence on adventitious sprouting in *P. pashia*. That the effect is greatest for 0.1 mg/ml Stik is in agreement with the findings of Singh and Paliwal[8], who showed that 100 and 500 ppm (i.e. 0.1 and 0.5 mg/ml) Stik hastened sprouting and subsequent shoot growth in stem cuttings of *Aerva sanguinolenta*. Sharma *et al.*[9] have reported a similar effect of Stik on the lenticel meristem in *Sapium insigne*. Eliason and Areblad[7] reported that IAA at 100 μM usually increased the number of roots, although variable results were obtained with IAA concentrations in light-grown stem cuttings of *Pisum sativum* cv. Weibull's Marina.

As far as the physiomorphological effect is concerned, it has been concluded that the stimulation of sprouting caused by Stik treatment is probably a consequence of conversion of starch to soluble sugars, which thus became available for active cell division and subsequent elongation, as has been postulated by Nanda *et al.*[10] The rapid degradation of starch by auxins is brought about by an increase in the activity of hydrolytic enzymes[11-13]. Altmann and Wareing[14] have also shown that IAA has a definite impact on sugar accumulation; however, according to them the relationship between auxins and carbohydrates is complex, since the auxins may influence basal carbohydrate accumulation directly as well as via the sink arising from the auxin-