RESEARCH PUBLICATIONS
SECTION VIII

MORPHOGENESIS, PLANT TISSUE CULTURE AND BIOTECHNOLOGY

VIII-1 IN VITRO ANther CULTURE STUDIES IN NEEM (AZADIRACHTA INdICA A. JUSS). K. JANARDHANAN, Seed Technology Laboratory Botany Department Bharathiar University Coimbatore - 641 046 (T. N.).

The floral buds (2 mm long) from elite neem tree were collected. The dissected and excised anthers were surface sterilised with 0.1% (W/V) mercuric chloride for 10 min. followed by tween 20 at a concentration of 0.05% (V/V). They were thoroughly washed with sterile Millipore water. Anther explants were planted onto Nitsch & Nitsch (N&N) (1969) basal medium supplemented with 0.2 mg/1 BAP and different concentrations of NAA or IAA or 2,4 - D for callus induction. Profuse callusing from anthers occurred when anthers were cultured on N&N basal medium supplemented with 0.2 mg/1 BAP and 1.5 - 2.0 mg/1 NAA in 4 weeks after culture.

Shoot bud induction was achieved by subculturing neem calli on Murashige & Skoog's (MS) (1962) medium supplemented with different concentration of BAP, KN and NAA or by supplementing the basal medium with 0.2 mg/1 BAP + 0.1 mg/1 KN along with different concentrations of Ga3. Induction of maximum number of shoots was recorded on the aforesaid basal medium supplemented with 1.0 mg/1 BAP; 0.5- 0.75 mg/1 KN and 0.1 mg/1 NAA. Similarly the aforesaid basal medium when supplemented with 0.2 mg/1 BAP, 0.1 mg/1 KN and 1.0 - 1.5 mg/1 Ga3 also registered the formation of maximum number of shoots 8 weeks after subculture. Root induction from the regenerated shoots was effected by subculturing the dissected shoot buds on the aforesaid basal medium supplemented with 1.0- 2.0 mg/1 IAA in four weeks time. Thus entire neem plantlets were restored in a stepwise sequential in vitro micropropagation technology.


A preliminary survey of lemnoids in Banda district has been made
during present investigation. Four genera spread over 4 species were
collected during present investigation. Species of *Spirodella* followed by
*Lemma* was dominant. *Lemma paucicostata, walsia arrhiza, Spirodella*
polyrhiza and *Azolla pinnata* were recorded.

Biotechnological research involve converting discoveries in new
biology into agricultural applications. While genetic research has led to
production of high yielding varieties of crops, there has been a need for
supplementing requirements to improve crop productivity. Balance supply
of nutrition and hormonal control of growth in unison are unarrivable for
strong agricultural economy.

Utilization of growth promoting substances present in these aquatic
plant extracts are known to boost crop productivity. Present investigation
has been made explore the possibilities of utilization of these duck-weeds to
boost the productivity of different crops growing in Banda district as well as
Bundelkhand region.

VIII-3 *IN VITRO PROPAGATION OF GYMNEMA SYLVESTRE R.Br.
THROUGH LONG TERM CALLUS CULTURE. SANTI SAHOO AND
RITARANI DAS, P. G. Department of Botany, Utkal University.*

In recent years the technique of tissue and organ culture has been
effectively used in the mass multiplication of a number of plants of medicinal
values. However, in a few cases the leaf material has been used as the source
of tissue. *Gymnema sylvestre* R. Br. is a woody climber and is highly
stomachic, stimulant, laxative and diuretic. The leaves have been used as a
remedy for diabetes. The leaves of this plant contain gymnemic acid
hetriacontane, pentatriacontane, resins, tartaric acid, formic acid, butyric
acid, anthraquinone derivaties and insitol, this plant is currently considered
to be a commercial source of gymnemic acid. Vegetative propagation of this
plant is not so easily possible. Depending upon the importance of this plant, a
large scale production of this plant is necessary. The present investigation
was therefore, taken to develop an efficient tissue culture method for
plantlet formation from leaf tissues of *G. sylvestre*.

*In vitro* clonal propagation of *G. sylvestre* an important medicinal
plant has been achieved through callus culture. Callusing was achieved on
RT medium which was found to be an excellent callusing medium for this
including plant height number of branches, number of leaves, leaf areas were recorded at fortnightly intervals. Comparisons were also made with respect to dry matter increase RGR, NAR and leaf area increase on weekly basis. The plants of C. verrucosa were with consistent higher number of branches and plant height. The dry matter accumulation and leaf area increase also showed similar trend in both the species. However, in later stages these values showed enhancement in C. grahmi a corroboring the biennial nature of the plant. The NAR corresponded the RGR in both the species while the former showed typical upswinging in the later stages of growth. Crotalana verrucosa on the other hand displayed the decreasing trend of RGR and NAR with aging days. It flowered over 70 days. While the other species remained vegetative even after 84 days of sowing.


Biotechnological research involve converting discoveries in new biology into agricultural applications. While genetic research has led to production of high yielding varieties of crops, there has been a need for supplementing requirements to improve crop productivity. Balance supply of nutrition and hormonal control of growth in unison are unarrivable for strong agricultural economy.

Influence of different concentrations (2 and 1 percent) of water and ether extract suspended in water on pottash content of wheat has been studied by pre-soaking seed treatment. Effect of Spirodeella polyrhiza extracts on pottash content of wheat have shown promising results. Twelve hours treatment with 1 percent ether-water extract exercises maximum increase in pottash content of wheat. Observed increases in pottash content of grain are of considerable significance towards edible value and roughage quality of wheat and appears to be at the expense of other constituents of lesser commercial significance. Results obtained are statistically significant.

Utilization of growth promoting substances present in these aquatic plant extracts are known to boost crop productivity. Present investigation has been made explore the possibilities of utilisation of these duck-weed to boost the productivity of different crop growing in Banda district as well as Bundelkhand region.

VII-12 EFFECT OF UV-B RADIATION ON SEEDLING GROWTH AND CHLOROPHYLL CONTENT OF VIGNA SPECIES, G.K. DHINGRA* & V.K. JAIN, Department of Botany, Govt. P.G. College, Rishikesh-249 201.

*Govt. P.G. College, Uttarkashi-249 193 (Uttaranchal).

Effect of daily exposure of UV-B radiation on seed germination, seedling growth and chlorophyll development in mung and urd bean grown in Petridishes have been observed in the present investigations. Data shows that seed germination was least
INFLUENCE OF *Spirodella polyrhiza* EXTRACTS ON JUVENILE SEEDLING GROWTH AND DEVELOPMENT OF WHEAT

A.K.TRIPATHI AND A.K.AWASTHI
Department of Botany Pt. Jawahar Lal Nehru Post Graduate College, Banda - 210001 (U.P)

ABSTRACT

Effect of water and ether extracts suspended in water of *Spirodella polyrhiza* on juvenile seedling growth of wheat variety "U.P. - 2338" has been studied by pre-soaking seed treatment. Results suggest that growth and development of both root and shoot is promoted following pre-soaking seed treatment with 1.0 percent extract. Observations are proven with possibilities of better prospective yield of wheat in treated plants. Results obtained are statistically significant.

Key words: (*Spirodella polyrhiza* wheat, growth and development)

INTRODUCTION

There is evidence of growth promoting substances in extracts of variety of plant materials (Mowat\(^8\) Maheshwari and Bhatia\(^7\) Shukla and Gupta\(^1\) Pronano and Greene\(^10\) Bhalla\(^2\), Gupta and Agarwal\(^12\), Pandey\(^9\), Shukla\(^14, 16\)). Such plant extracts have been utilized to boost growth, development and yield of various crop plants. The topic on Agricultural application of plant extracts for improved yield and altering quality of produce has been reviewed earlier (Shukla\(^15, 16\)).

Lemnoids are known for their wide spread occurrence in south-east Asia. *S. polyrhiza* particularly exhibits massive infestation at Banda. Present investigation deals with effects of *S. polyrhiza* extracts on growth and development of wheat seedling. *S. polyrhiza* was particularly chosen as experimental material for obtaining extract in view of its availability in large quantities in the area and homogeneity of structure for uniform extraction.

MATERIAL AND METHODS

Seeds of wheat variety "U.P. - 2338" were obtained from Economic Botanist,
C.S. Azad University of Agriculture and Technology, Kanpur. Seeds of approximately same size and weight were selected for experiments.

*S. polyrhiza* was obtained from nature and grown in laboratory under *in-vitro* cultural conditions. Healthy plants of *S. polyrhiza* were collected for obtaining water and ether extracts. Five ml. of *S. polyrhiza* material by volume was ground in ether or water in a porcelain mortar. In case of water extracts sufficient water was added to give 100 ml of 5.0 percent extract. In ether extracts ether was allowed to evaporate and suspension was made in 100 ml distilled water. Two and 1.0 percent extracts were made further dilution with distilled water. Fifty seeds were soaked in sterilized petridishes in different concentrations (1.0, 2.0 and 5.0 percent) of water and ether extracts of *S. polyrhiza* and distilled water (Control) for 6, 12, and 24 hours. Desired concentration used on experiments to study various parameters are specified separately below. Immediately after the soaking period seedlings were grown in test tubes filled with distilled water on equal sized filter papers following Garrard's Technique. The experiments were carried out at temperature of 20° - 24°C, the normal temperature range of crop in nature. Observation were made 48, 72 and 96 hours on length of main root, number of secondary roots and length of shoot. Seedlings were preserved as herbarium specimens.

**OBSERVATION**

A perusal of table I, II and III indicates that juvenile seedling growth of wheat plants show that out of various concentrations (1.0, 2.0 and 5.0 percent) of extracts tried, 1.0 percent extracts exhibits all round maximum beneficial growth. Length of primary root, number of secondary roots and length of plumule exhibit marked increase with 1.0 percent extracts (except 6 hrs soaking with water extract where 2.0 percent is more effective).

**DISCUSSION**

Response of root and shoot growth of wheat seedlings to pre-soaking seed treatment with *S. polyrhiza* extracts exhibits marked beneficial effect. Treatments with 1.0 percent extract exhibits maximum effects on length of primary root, number of secondary root and length of plumule (except 6 hrs. soaking with water extract where 2.0 percent is more effective).
There is uniform all round maximum effectiveness of extracts in concentration of 2.0 percent ether and 1.0 percent water in 6 hrs. 1.0 percent ether and 1.0 percent in 12 hrs and 1.0 percent ether and 1.0 percent water in 24 hrs of S. polyrhiza. Evidently ether extracts of S. polyrhiza are more effective than ether extracts.

Increase under 6 hrs treatments in length of primary root, number of secondary roots and length of plumule increased 23.76, 55.76 and 85.74 percent with 1.0 percent ether extract respectively. However, increase in length of primary root, number of secondary roots and length of plumule were found to be 34.61, 64.09 and 89.67 percent with 2.0 percent water extract respectively during present investigation on expiry of experiments after 96 hrs. Increase under 12 hrs. treatments in length of primary root, number of secondary roots and length of plumule were increased 29.17, 40.93, and 75.12 percent with 1.0 percent ether extract respectively. Increases in length of primary root, number of secondary roots and length of plumule were found to be 26.93, 55.48 and 67.35 percent with 1.0 percent water extract respectively during present investigation on expiry of experiment after 96 hrs. Increase under 24 hrs treatments in length of primary root, number of secondary roots and length of plumule increased 46.93, 42.95 and 91.90 percent with 1.0 percent ether extract respectively, but increase in length of primary root number of secondary roots and length of plumule were found to be 39.79, 60.64 and 96.94 percent water extract respectively.

Observation emphasize that in general effects of 12.0 hrs treatment with both ether and water extract is better as compared to 6 and 24 hrs treatments. Stimulatory effect of treatments gradually declines with pre-soaking seed treatment periods and 24 hrs.

There is existence of extensive literature on effect of natural plant extracts on variety of plants (Shukla and Gupta\textsuperscript{11}, Shukla\textsuperscript{12, 13, 14} Shukla and Shukla\textsuperscript{15}) Beneficial effect of naturally occurring growth substances of various plant extract like Phormidium foveolarum and P. tenue on rice (Shukla\textsuperscript{12, 16}) P. foveolarum on Phaseolus aurens (Gupta and Gupta\textsuperscript{4}), Vigna catjang (Gupta and Gupta\textsuperscript{5}); wheat (Kushwaha and Gupta\textsuperscript{7}), Spirodella polyrhiza extracts on barley (Pandey\textsuperscript{9}) and Wolffia arrhiza extract on wheat (Awasth\textsuperscript{1}). Present observations are in general agreement with previous
### TABLE 1: Effect of 6 hours Pre-soaking Seed Treatment with *Spirodella polyrhiza* Extracts on Juvenile Seedling growth.

**ETHER EXTRACT**

<table>
<thead>
<tr>
<th>Age of Seedlings</th>
<th>Length of Primary root in CM</th>
<th>No. of Secondary Roots</th>
<th>Length of Plumule in CM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>48 HRS</td>
<td>2.56</td>
<td>4.03</td>
<td>3.42</td>
</tr>
<tr>
<td>72 HRS</td>
<td>4.73</td>
<td>6.51</td>
<td>5.66</td>
</tr>
<tr>
<td>96 HRS</td>
<td>5.85</td>
<td>7.24</td>
<td>6.93</td>
</tr>
</tbody>
</table>

C.D. = 0.25
Difference :96 HRS
1% - Control = 1.39

C.D. = 0.39
Difference :96 HRS
1% - Control = 1.79

C.D. = 1.55
Difference :96 HRS
1% - Control = 4.33

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**WATER EXTRACT**

<table>
<thead>
<tr>
<th>Age of Seedlings</th>
<th>Length of Primary root in CM</th>
<th>No. of Secondary Roots</th>
<th>Length of Plumule in CM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>48 HRS</td>
<td>2.12</td>
<td>3.10</td>
<td>3.91</td>
</tr>
<tr>
<td>72 HRS</td>
<td>4.31</td>
<td>5.24</td>
<td>6.33</td>
</tr>
<tr>
<td>96 HRS</td>
<td>5.20</td>
<td>6.62</td>
<td>7.00</td>
</tr>
</tbody>
</table>

C.D. = 0.58
Difference :96 HRS
2% - Control = 1.80

C.D. = 0.31
Difference :96 HRS
2% - Control = 1.91

C.D. = 1.53
Difference :96 HRS
2% - Control = 4.43

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Abbreviations used: C = Control. C.D. = critical difference.

### TABLE 2: Effect of 12 hours Pre-soaking Seed Treatment with *Spirodella polyrhiza* Extracts on Juvenile Seedling growth.

**ETHER EXTRACT**

<table>
<thead>
<tr>
<th>Age of Seedlings</th>
<th>Length of Primary root in CM</th>
<th>No. of Secondary Roots</th>
<th>Length of Plumule in CM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>48 HRS</td>
<td>2.93</td>
<td>4.33</td>
<td>3.86</td>
</tr>
<tr>
<td>72 HRS</td>
<td>6.86</td>
<td>9.15</td>
<td>8.75</td>
</tr>
<tr>
<td>96 HRS</td>
<td>9.22</td>
<td>11.91</td>
<td>10.53</td>
</tr>
</tbody>
</table>

C.D. = 0.74
Difference :96 HRS
1% - Control = 2.69

C.D. = 0.37
Difference :96 HRS
1% - Control = 1.49

C.D. = 1.67
Difference :96 HRS
1% - Control = 4.53
### WATER EXTRACT

<table>
<thead>
<tr>
<th>Age of Seedlings</th>
<th>Length of Primary root in CM</th>
<th>No. of Secondary Roots</th>
<th>Length of Plumule in CM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>48 HRS</td>
<td>2.81</td>
<td>4.12</td>
<td>3.40</td>
</tr>
<tr>
<td>72 HRS</td>
<td>6.53</td>
<td>8.78</td>
<td>7.87</td>
</tr>
<tr>
<td>96 HRS</td>
<td>8.95</td>
<td>11.36</td>
<td>10.00</td>
</tr>
</tbody>
</table>

C.D. = 0.68  
Difference : 96 HRS  
1% - Control = 2.41

Abbreviations used: C = Control, C.D. = critical difference.

### ETHER EXTRACT

<table>
<thead>
<tr>
<th>Age of Seedlings</th>
<th>Length of Primary root in CM</th>
<th>No. of Secondary Roots</th>
<th>Length of Plumule in CM</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>C</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>48 HRS</td>
<td>2.05</td>
<td>3.58</td>
<td>2.21</td>
</tr>
<tr>
<td>72 HRS</td>
<td>4.12</td>
<td>6.00</td>
<td>5.48</td>
</tr>
<tr>
<td>96 HRS</td>
<td>4.88</td>
<td>7.17</td>
<td>6.31</td>
</tr>
</tbody>
</table>

C.D. = 0.64  
Difference : 96 HRS  
1% - Control = 2.29

Abbreviations used: C = Control, C.D. = critical difference.

### WATER EXTRACT

<table>
<thead>
<tr>
<th>Age of Seedlings</th>
<th>Length of Primary root in CM</th>
<th>No. of Secondary Roots</th>
<th>Length of Plumule in CM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>48 HRS</td>
<td>2.00</td>
<td>3.23</td>
<td>2.64</td>
</tr>
<tr>
<td>72 HRS</td>
<td>3.71</td>
<td>5.97</td>
<td>4.88</td>
</tr>
<tr>
<td>96 HRS</td>
<td>4.90</td>
<td>6.85</td>
<td>5.76</td>
</tr>
</tbody>
</table>

C.D. = 0.45  
Difference : 96 HRS  
1% - Control = 1.95

Abbreviations used: C = Control, C.D. = critical difference.
reports. They also exhibit a similar trend of promotion in growth and developmental wheat seedlings following treatments with S. polyrhiza extracts. Present findings are suggestive of better crop prospects should these extracts are applied in agriculture of wheat crop.

REFERENCE


The paper is accepted for publication in The Journal of the Indian Botanical Society - 2001
EFFECT OF *Spirodella polyrhiza* EXTRACTS ON STOMATAL AND EPIDERMAL DEVELOPMENT IN WHEAT LEAVES

A.K.TRIPATHI AND A.K.AWASTHI

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Banda - 210001 (U.P.)

ABSTRACT

The influence of different concentrations (1 and 2 percent) of water and ether extracts of *Spirodella polyrhiza*, suspended in water on stomatal and epidermal development of *Triticum aestivum* leaves has been studied after pre-soaking the seeds. Treatments for 6 hrs with one per cent water extracts gave maximum increase in number of epidermal cells, number of stomata, length of epidermal cell, breadth of epidermal cell, perimeter of stomatal pore, length of guard cells and breadth of guard cells. The results demonstrate an overall change in morpho-anatomy of the leaf, acquiring better adaptability for gaseous exchanges vital for photosynthetic activity. The results were statistically significant.

INTRODUCTION

There has been growing concern about rapid spread and luxuriant infestation of duck-weeds in tropical and sub-tropical regions of the world. Naturally, such massive growth of duck-weeds is proven with possibility how best it could be harnest to benefit mankind. *S. polyrhiza* of the duck-weeds explored for its extracts to investigate their response to synthesis in wheat variety 'U.P.-2338'. Wheat production is of pivotal importance to meet problem of malnutrition in both human beings and cattle. There is evidence for growth-promoting and inhibitory substances in extracts of a variety of plant materials (Matzger and Zeevaart, 1980; Albone et al. 1984; Pontovich et al., 1984; Ahokas, 1985; Gaskin et al., 1985; Janaki and William, 1986; James and Marcia, 1986 and Prudence et al., 1986). The influence of growth substances on anatomical structure has also been reported by Torrey, 1953; Kennedy and Farrar, 1965; Cronshaw and Morey, 1965; Morey and Cronshaw, 1966; Weston and Thomas, 1980; Ting and Wren, 1980; Saks et al., 1984; Vreugdenhill et al.,
1984. However, there is little information on the influence of plant extracts on leaf anatomy of wheat. *S. polyrhiza* was chosen for extracting, since there is evidence for the presence of growth regulating properties in aquatic weeds (Sircar and Kundu, 1959, 1960; Mukherjee et al., 1964; Nagar and Saha, 1985 and Fujioka et al., 1986) and homogeneity of structure for uniform extraction.

MATERIALS AND METHODS

Seeds of the wheat (*Triticum, aestivum*) variety U.P.-2338 were obtained from Economic Botanist, C. S. Azad University of Agriculture and Technology, Kanpur, India. Seeds of approximately the same size and weight were selected for experiments.

* Spirodella polyrhiza was obtained from nature and grown in the laboratory under *in vitro* cultural conditions (Pandey, 1979). The extraction of *S. polyrhiza* were made in water or ether from healthy plants. As ether is injurious to plant growth, it was allowed to evaporate and the growth promoting substances were suspended in water. Five millilitre of *S. polyrhiza* by volume was ground in a clean porcelain mortar with 10 ml water or ether. In case of water extract sufficient distilled water was added to make it 100 ml to have a five percent extract. One and 2 per cent extracts were made by further dilution with distilled water. In case of ether extract ether was first allowed to evaporate and the suspension was than made to 100 ml in distilled water. One 2 and 5 per cent extracts were made by further dilution with distilled water. Fifty seeds were soaked for 6, 12 and 24 h in sterilized petridishes in different concentrations (1, 2 and 5 per cent) of water and ether extracts of *S. polyrhiza*, and distilled water (control).

Effect of 6, 12 and 24 h pre-soaking seed treatment with 1 per cent water and 2 per cent ether extracts of *S. polyrhiza* on stomatal and epidermal development of wheat seedlings, variety "U.P.-2338" was studied following technique suggested by Shukla (1967). Treated wheat seedlings were allowed to grow for 144 h. Second leaf of seedlings from base in different treatment was collected and preserved in alcohol (Lloyd, 1908). The stomatal and epidermal studies were made from peelings of leaves. Both upper and lower epidermal peelings were taken out and stained preparations were observed microscopically. One per cent water and 2 per cent ether extracts were chosen for observing anatomical response of wheat leaves because out
of various concentrations used to study seedling growth, these concentrations were
found to be beneficial to the maximum extent. Observation on number of stomata,
perimeter of single stomatal opening, number of epidermal cells, length of epidermal
cells, and length and breadth of guard cells were made in an area of 1984 sq m of
leaf peelings. Average of 25 replicates were taken into consideration.

The data was analysed statistically following analysis of variance method at
5 per cent error probability for testing the significance of the effect of treatments.
Results of statistical analysis are entered in respective observation tables.

**OBSERVATIONS**

An examination of Table-1 shows that number of epidermal cells per
microscopic field, number of stomata per microscopic field, length of epidermal cell,
breadth of epidermal cell, perimeter of stomatal pore, length of guard cells and
breadth of guard cells increased 14.00, 60.73, 16.02, 15.18, 16.71, 10.90 and 42.40;
9.80, 46.52, 46.21, 19.50, 24.02, 11.01 and 24.59, 6.99, 75.58, 9.61, 12.08, 13.28,
11.59 and 40.58 per cent over control with ether extract and 4.75, 28.86, 5.34, 4.56,
10.05, 6.39 and 22.24: 4.78, 21.66, 31.53, 11.78, 12.49, 5.83 and 12.82; 3.54,
70.50, 7.13, 3.59, 3.90, 4.19 and 17.69 percent over control with water extract
under 6, 12 and 24 hrs treatments respectively on upper epidermis of leaves.

A perusal of Table-ll exhibits that number of epidermal cells per microscopic
field, number of stomata, length of epidermal cell, breadth of epidermal cell, perimeter
of stomatal pore, length of guard cells and breadth of guard cells increased 9.16,
21.45, 11.39, 18.59, 3.09, 5.24 and 25.68; 10.66, 47.52, 12.77 8.19, 15.34, 7.90,
and 30.23; 10.34, 79.66, 12.38, 15.87, 4.38, 2.78 and 22.38 per cent over control
with ether extract and 8.21, 17.43, 9.18, 12.10, 1.51, 4.05 and 18.84; 5.68, 22.98,
10.31, 6.19, 7.91, 2.93 and 20.10: 5.47, 74.15, 10.00, 7.28, 2.98, 1.59 and 20.07
per cent over control with water extract under 6, 12 and 24 hrs treatments respectively
on lower epidermis of leaves.

**DISCUSSION**

Exhaustive literature on utilisation of algal extracts of *Phormidium
foveolarum* in agriculture of rice (Gupta and Shukla, 1964, 1967, 1969; Shukla and
Gupta, 1967; Shukla, 1968, 1975 a); wheat (Kushwaha and Gupta, 1970 a, 1970
Vigna catzang (Gupta and Gupta, 1970, 1972, 1973) and Phaseolus aureus (Gupta and Gupta, 1972) is available. Likewise, stimulation in vegetative growth and yield of rice following treatments with water hyacinth extracts has also been reported (Sircar, 1963). Influence of Lemna paucicostata manure and spraying with its extracts on Hordeum Vulgare was explored by Pandey (1979) and the study revealed significant effects on fresh dry matter production, yield, ascorbic acid, catalase, chlorophyll and epidermal structure of plants.

The physiological peculiarities of plants in general and rice in particular (Sircar, 1958) are known to possess different auxin levels at various sites which appears to control growth and development pattern in various parts of the plants. A high IAA content of endosperm regulates germination and seedling growth in rice (Sircar and Das, 1954). It has been suggested that auxin level in rice occurs in two parts. A bulk of auxin remains in inactive form in vacuole and active auxin part below suboptimal concentration is found at the sites of growth to bring about stimulation. Exogenous supplies of other growth regulators, sets in a competition between native IAA and exogenous growth regulators which displaces native auxin from its natural site of action leading to higher concentration of free auxin to exercise stimulated growth (Sircar, 1958).

It may be suggested that a similar auxin level controlled mechanism as referred to earlier in case of rice may be operative in wheat under exogenous supply of growth substances present in S. polyrhiza extracts.

The stomata are principal portals through which gaseous exchanges take place between the intercellular spaces and surrounding atmosphere. The efficiency of stomatal apparatus in controlling gaseous exchanges of the plants was extensively studied by Brown and Escombe (1900), who have pointed out that the rate of diffusion through small openings (like stomata) in a given period of time is proportional to the perimeter and not to the area of pore. The greater the perimeter the more rapid is the rate of diffusion. Earlier observations of Shukla (1967) revealed that application of 1 per cent extract of Phormidium foveolarum reduces the size of stomata in treated rice plants but increase their number and perimeter. Consequently, it was suggested that there would be more rapid diffusion of carbondioxidc in the
leaves of treated rice plants. Similar influence of algal extracts on stomatal and epidermal development of wheat leaves (Shukla, 1975 b) and synthetic growth substances on maize leaves has been recorded earlier (Shukla and Shukla, 1975).

The crude fresh extract of *S. polyrhiza* plants applied to wheat crop appears to contain growth substances. Interestingly containing such a mixture of substances that provide an ideal blending of growth factors sufficiently endowed to give general boost to the crop expressed in terms of altering morpho-anatomy of wheat crop.

Present findings emphasized the significance of *S. polyrhiza* infestation, and how best they could be utilized for obtaining extracts which possess tremendous capacity to stimulate stomatal and epidermal development. The stomatal and epidermal structure also acquired better adoptability for gaseous exchanges vital for photosynthetic activity. This may partly explain the beneficial effect of *S. polyrhiza* extracts on the growth of wheat plant.

The investigation opens up wide vistas for further exploration and enquiry in intricate aspects concerned with utilizing the technology with success in future with new influx of varieties to boost wheat productivity in time and space. Implementation of these growth promoting substances containing extracts wheat agriculture to meet human hunger and malnutrition is only possible when these experimental findings are wedded to practice.

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STUDIES ON EFFECT OF Spirodella polyrhiza EXTRACTS
ON MORPHO-ANATOMY OF WHEAT STEM

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ABSTRACT

Influence of different concentrations (2 and 1 percent) of water and ether extracts suspended in water on anatomical structure of stem of wheat has been studied by pre-soaking seed treatment. Effect of S. polyrhiza extracts on stem anatomy of wheat have shown promising results. Six twelve and Twentyfour hrs treatments with ether extract exercises maximum increase in diameter of xylem, phloem and number of vascular bundles in stem. Results emphasize an overall change in morpho-anatomy of stem to provide better conductions and facilitate better vegetative growth and yield. Results obtained are statistically significant.

Tables : 01  figures : 00  References : 37

Key Words : Wheat, Spirodella polyrhiza, Stem Anatomy

INTRODUCTION

dearth of literature concerned with the influence of extracts of plant material to the anatomical structure. (Awasthi and Shukla, 1986, 1989) Present investigation deals with effects of *S. polyrhiza* extracts on stem anatomy of wheat. *S. polyrhiza* was particularly chosen as experimental material for obtaining extracts in view of evidence for the presence of growth regulating properties in the aquatic weeds (Sircar and Kundu, 1959; 1960; Mukherjee et. al., 1964, Nagar and Saha, 1985 and Fujioka et. al., 1986) and homogeneity of structure for uniform extraction.

**MATERIAL AND METHODS**

Seeds of wheat variety 'U.P.- 2338' were obtained from Economic Botanist, C. S. Azad University of Agriculture and Technology, Kanpur, India. Seeds of approximately same size and weight were selected for experiments.

*S. polyrhiza* was obtained from nature and grown in laboratory under in vitro cultural conditions. Healthy plants of *S. polyrhiza* were collected for obtaining water and ether extracts. The extractions of *S. polyrhiza* were made in water or ether. As ether is injurious to plant growth it was allowed to evaporate and growth promoting substances were suspended in water. Five millilitre of *S. polyrhiza* by volume was taken and ground in a clean porcelain mortar with water or ether. In case of water extract sufficient distilled water was added to make it 100 ml and get five per cent extract. One and 2 per cent extracts were made by further dilutions with distilled water. In case of ether extract ether was first allowed to evaporate and then suspension was made in 100 ml in distilled water. One, 2 and 5 per cent extracts were made by further dilution with distilled water. Fifty seeds were soaked in sterilized petridishes in different concentrations (1,2 and 5 percent) of water and ether extracts of *S. polyrhiza*, and distilled water (control) for 6, 12 and 24 hrs.

The effect of treatments was studied under field conditions in the garden beds laid for specific purpose with dimensions 8 feet in breadth and 10 feet in length. Each bed was sown with 3 rows containing 9 seeds spaced 25 cm in rows 60 cm apart. Thus total number of plants grown in each bed were 27 out of which 25 were selected for observations. Two beds of each treatments and normal untreated control were laid to raise 50 replicates. Seeds of wheat variety 'U.P.-2338' 1 percent water and 2 per cent ether extracts were chosen for observing effects under normal field
conditions as out of various concentrations used to study seedling growth, these concentrations were found to be beneficial to the maximum extent and therefore, it was thought to study sustained effect of such treatments on subsequent nature of wheat stem. Garden beds were prepared after ploughing the area and mixing adequate amount of manure in ratio of 3 parts of soil and 1 part of cowdung manure in upper crust of soil. Seeds soaked in distilled water were similarly sown and served as control. The matured crop was harvested after 105 days and material of stem was cut carefully from both treatments (1 per cent water and 2 per cent ether) and control. Stem pieces of 2 cm were collected from 5 cm below the top of the plant. Such materials of each treatment were preserved in formalin aceto-alcohol, in a mixture containing 90 ml of 70 percent ethyl alcohol, 5 ml. glacial acetic acid and 5 ml formalin. The material was then dehydrated and was microtomed using senior Rotary Microtome Model MT 1090 A. Slides of materials prepared were stained in safranin and fast green following Johansen’s (1940) technique. Observations on diameter of stem, number of vascular bundles, diameter of xylem and phloem and diameter of xylem were recorded and average of 50 replicates taken into consideration. Results obtained were statistically analysed following “Analysis of variance” method to pinpoint significance of treatments at 5 per cent error probability.

**OBSERVATIONS**

Observations on diameter of stem, number of vascular bundles, diameter of xylem and phloem and diameter of xylem tissue have been recorded in Table 1. A perusal of data entered in Table shows that diameter of xylem tissue, diameter of phloem tissue, diameter of stem, diameter of metaxylem, diameter of protoxylem and number of vascular bundles increased 23.02, 19.88, 13.86, 27.97, 7.66, and 39.90; 24.00, 23.49, 11.55, 16.80, 8.65 and 27.87; 26.14, 31.93, 39.35, 33.52, 17.36 and 40.71 percent over control with ether extract (1 per cent) under 6, 12 and 24 hrs treatments respectively in stems. Diameter of xylem tissue, diameter of phloem tissue, diameter of stem, diameter of metaxylem, diameter of protoxylem and number of vascular bundles increased 11.35, 5.50, 7.80, 22.38, 3.70, and 22.72; 8.97, 7.90, 10.99, 11.81, 2.68, and 18.06; 16.48, 16.32, 34.81, 18.29, 14.11 and 19.56 per cent over control in 6, 12 and 24 hrs treatments respectively in stems with water.
DISCUSSION

A perusal of results on the effect of *S. polyrhiza* extracts on diameter of xylem, phloem and number of vascular bundles shows a marked alteration. The xylem-phloem and ground tissue ratio is concomittantly effected. The increase in xylem-phloem tissue appears to be at the expense of cortex and ground tissue.

The number of tracheary elements found in the tension wood induced by DNP in some seedlings are either equivalent or in others slightly reduced, relative to the number of tracheary elements present in xylem formed before treatment. In the *Acer rubrum* system (Morey, 1968a; 1968b) it is probable that the relative frequency at which tracheary elements are initiated from the fusiform initials related to the level of auxin in the system below the DNP treatment site where tracheary elements are initiated from the deviding intitials or adjacent to them. It seems inconsistent, on the other hand that the capacity of DNP to induce the formation of tension wood in the same region of the stem is explained in terms of developmental response to auxin deficiency. However, the cambial derivatives undergoing secondary wall development namely the xylem element in the wall thickening phase of development are segregated from the cambial initials by more or less arbitrary zone of cells in which the walls are expended by surface growth (Morey and Cronshaw, 1966). In this regard DNP may be more effective in lowering the auxin level in the cetripetal zone of the stem than in the peripheral peristematic region.

This synoptical background about development of tracheids is clearly indicative of the fact that development of xylem is linked with auxin level in stem. Auxin deficiency stimulates development of xylem. Exogenous supplies of certain growth substances blocked polar transport of auxin in area just above the region of blockade (Cronshaw and Morey, 1965; Jackson and Stead, 1984). During present investigation exogenous supply of growth substances in extracts of *S. polyrhiza* administered through pre-soaking seed treatment appear to set in some kind of competition with the endogenous auxin levels and displaces auxin through polar transport to the extremities of stem to initiate its apical growth, and in the process create conditions of auxin deficiency in the older region of the organs, thereby stimulating
development of xylem in the stem. This may explain the increased formation of xylem, phloem and ground tissue observed during present investigation.

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RESPONSE OF WHEAT ROOT ANATOMY TO Spirodella polyrhiza EXTRACTS

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ABSTRACT

Influence of water and ether extracts suspended in water of Spirodella polyrhiza on root anatomy of wheat has been studied by presoaking seed treatment. There is a uniform alround maximum effectiveness of 1 per cent ether extracts of S. polyrhiza. Six, twelve and twenty four hours treatments with ether extract of S. polyrhiza exercised maximum increase in xylem, phloem and ground tissue to the maximum extent. Results obtained were found to be statistically significant.

Tables : 01
figures : 00
References : 41
Key Words : Wheat, Spirodella polyrhiza, Root Anatomy

INTRODUCTION


Despite abundance of literature concerned with anatomy of various plants there is dearth of literature on influence of growth promoting substances vis-a-vis anatomy of plant systems. However, works of Torrey (1953), Roberts (1960), Kennedy and Farrar (1965), Cronshaw and Morey (1965) and Morey and Cronshaw (1966)
Awasthi and Shukla (1986, 1989) on influence of growth substances with reference to the anatomical structure have been reported elsewhere.

Lemnoids are known for their wide spread in South—East Asia. *S. polyrhiza* particularly exhibits massive infestation in Banda. Present investigation deals with effects of *S. polyrhiza* extracts on anatomy of wheat root. *S. polyrhiza* was particularly chosen as experimental material for obtaining extracts in view of the evidences for presence of growth regulating properties of the aquatic weed (Sircar and Kundu, 1959, 1960; Mukherjee et al., 1964) and homogeneity of structure for uniform extraction.

**MATERIAL AND METHODS**

Seeds of wheat variety "U.P.-2338" were obtained from Economic Botanist, C.S. Azad University of Agriculture and Technology, Kanpur, India. Seeds of approximately same size and weight were selected for experiments.

*S. polyrhiza* was obtained from nature and grown in laboratory under *in vitro* cultural conditions. Healthy plants of *S. polyrhiza* were collected for obtaining water and ether extracts. The extractions of *S. polyrhiza* were made in water or ether. As ether is injurious to plant growth it was allowed to evaporate and growth promoting substances were suspended in water. Five millilitre of *S. polyrhiza* by volume was taken and ground in a clean porcelain mortar with water or ether. In case of water extract sufficient distilled water was added to make it 100 ml for obtaining 5 per cent extract. One and 2 per cent extracts were made by further dilutions with distilled water. In case of ether extract, ether was first allowed to evaporate and the suspension was then made to 100 ml in distilled water. One, 2 and 5 per cent extracts were made by further dilutions with distilled water. Fifty seeds were soaked in sterilized petri-dishes in different concentrations (1, 2 and 5 per cent) of water and ether extracts of *S. polyrhiza*, and distilled water (control) for 6,12 and 24 hours. Studies on effect of *S. polyrhiza*, extracts on anatomy of wheat plant, variety "U.P. - 2338" were made following usual techniques of fixation of desired parts of material. Material of root was cut carefully from both treated (1 per cent water and 2 per cent ether extracts of *S. polyrhiza*) and normal untreated plants served as control. Treated seeds were grown over filter papers following Garrard's
(1954) technique and roots were collected after seedlings obtained 144 hrs age. Seeds of wheat variety "U.P.-233S". 1 per cent water and 2 per cent ether extracts were chosen for observing anatomical responses of wheat roots, because out of various concentration used to study seedlings growth, these concentrations were found to be beneficial to the maximum extent and therefore, it was thought to study sustained effect of such treatments on subsequent nature of wheat roots. In view to obtain uniform effects of treatments pieces of roots were carefully selected from 5 mm below root-shoot transition zone. Such materials of each treatment were preserved in formalin aceto-alcohol (in mixture of 5 ml glacial acetic acid and 5 ml formalin). The material was then dehydrated and was micro-tomed using Senior Rotary Microtome Model MT 1090 A. Slides of materials prepared were stained in safranin and fast green following Johansen's (1940) technique. Observations on diameter of root, diameter of stele, diameter of vascular bundles, number of protoxylem, number of metaxylem and number of root hair were recorded. Results expressed are average of twenty five replicates.

**OBSERVATIONS**

An examination of data entered in Table—1 shows that 12 hrs treatment is effective to the maximum extent. Influence of ether extract in 6, 12 and 24 hrs are more pronounced. Increase in diameter of root, diameter of stele, diameter of vascular bundle, diameter of metaxylem, number of protoxylem and number of root hair increased 8.81, 17.65, 11.19, 11.15, 18.24 and 5.66: 13.57, 15.33, 10.44, 13.39, 13.46 and 13.05: 11.07, 14.26, 10.38, 16.47, 19.90 and 6.98 per cent over control in 6, 12 and 24 hrs treatments respectively in root with ether extracts. Likewise diameter of root, diameter of stele, diameter of vascular bundle, diameter of metaxylem, number of protoxylem and number of root hair increased 2.93, 4.72, 8.06, 5.56, 3.62 and 3.74: 7.04, 9.13, 7.10, 2.47, 3.48 and 6.05: 7.36, 5.20, 6.62, 8.39, 9.20 and 2.37: with water extract under 6, 12 and 24 hrs treatments respectively.

**DISCUSSION**

A perusal of results on the effect of *S. polyrhiza* extracts on diameter of xylem and phloem tissues and size of tracheids shows a marked alteration. The xylem-phloem and ground tissue ratio is concomitantly affected. The increase in
xylem-phloem tissue appears to be at the expense of cortex and ground tissue.

The number of tracheary elements found in the tension wood induced by DNP in some seedlings are either equivalent or in others slightly reduced interalia number of tracheary elements formed before treatment present in xylem. In *Acer rubrum* system (Morey, 1968 a, 1968 b) probability of relative frequency at which tracheary elements are initiated from the fusiform initials or adjacent to them has been suggested. It seems inconsistent, on the other hand that the capacity of DNP to induce the formation of tension wood in the same region of the stem is explained in terms of developmental response of auxin deficiency. However, the cambial derivatives undergoing secondary wall development, namely the xylem element in the wall thickening phase of development are segregated from the combial initials by more or less arbitrary zone of cells in which the walls are expanded by surface growth (Morey and Cronshaw, 1966). In this regard DNP may be more effective in lowering the auxin level in the centripetal zone of the stem than in the peripheral meristematic regions.

This synoptical background about development of tracheids in stem is clearly indicative of the fact that development of xylem is linked with auxin level. It appears that a similar auxin controlled trachied development in roots may be operative in wheat root. Auxin deficiency stimulates development of xylem. Exogenous supplies of certain growth substances blocked polar transport of auxin in area just above the region of blockade (Cronshaw and Morey, 1965; Jackson and Stead, 1984) During present investigation exogenous supply of growth substances in extracts of *S. polyrhziza* administered through pre-soaking seed treatments appear to set in some kind of competition with the endogenous auxin levels and displaces auxin through polar transport to the extremities of root and initiate its apical growth, and in the older regions of the organs, thereby stimulating development of xylem in the root.

There is evidence of gibberellin like substance in extracts of *Wolffia floridana* (Pieterse et al., 1971). The nature of growth promoting of *S. polyrhziza* was studied and it was found to contain a gibberellin-like growth factor. Interestingly, the effect of crude extract containing this growth factor in mixture of other plant constituents stimulated and altered growth and development of wheat plants to a greater extent.
than the gibberellin itself. Logically, it was considered more appropriate to use crude extract during present investigation rather than trying isolated growth factor of S. polyrhiza plants. Perhaps constituents other than gibberellin-like factor may be functioning like co-factors thereby exercising more pronounced effects. This may explain increased formation of xylem, phloem and diameter of tracheids, observed during present investigation.

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