EFFECT OF $\gamma$-IRRADIATION ON IMBIBITIONAL LEAKAGE, GERMINATION AND GROWTH OF WHEAT SEEDS

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ABSTRACT

Wheat seeds (cv. Inqalab) were treated with 0-100 Kr $\gamma$-radiation. The damage done to seeds was measured in terms of electrical conductivity (E.C.) of the leachate of imbibing seeds, the amounts and rates of leakage of inorganic and organic electrolytes in the leachates and the germination performance and growth of seedlings. Results demonstrate that $\gamma$-irradiation increased the E.C. of imbibing seeds compared with the un-irradiated control. Amounts of Na$^+$, K$^+$ and orthophosphate ions (Pi) increased in leachates of treated seeds in dose-dependent manner compared with the control. Similar patterns were observed when soluble sugars and total amino acid contents effluxed during initial hours of imbibition were measured. Leakage of these electrolytes had little effect on the number of seeds germinated, though the rates of germination were decreased in seeds treated with higher doses of radiation. Dose of $\gamma$-radiation at 1 Kr was stimulatory to root and shoot development compared with that of control whilst 0.5 Kr and radiation doses of 5-100 Kr were inhibitory for growth. Chlorophyll contents (a, b, total) in young leaves also increased but only in seeds treated with 1 Kr compared with that of control. The present data reveals variations in the permeability of various ions through the modified membrane system of irradiated seeds during early hours of germination and later during the active phase of seedling growth.

Key words: Electrical conductivity, germination, growth, $\gamma$-irradiation, imbibition, wheat seed.

INTRODUCTION

Seed maturation and desiccation is the final step in seed development. Membrane damage occurs during desiccation after seed development and during storage. When such a seed is allowed to imbibe, membrane integrity is restored during the initial minutes of imbibition (Simon, 1984). This membrane damage may be higher in older and low vigour seeds and cell deaths may take place due to rapid uptake of water in these seeds. It is this time during which solutes leak out of the cells. Greater the damage occurring to cell membranes due to stress treatments including $\gamma$-radiation, greater will be the electrolytes leakage during imbibition (Bewley and Black, 1986; Ashraf et al., 2000, 2001b).

Intact seeds do not leak or leak little in the surrounding medium. Water imbibition is always accompanied with the leakage of inorganic ions (mainly K$^+$ and phosphate ions), soluble sugars, organic acids, ions, amino acids and some proteins (Simon, 1984; Bewley and Black, 1986; Hussain, 1997; Ashraf et al., 2001a). The increase in electrical conductivity (E.C.) in leachates of imbibing seeds is due to the increased leakage of these electrolytes. High vigour seeds show decreased E.C. values and electrolytes leakage during initial hours of imbibition than the low vigour seeds because the membrane system of the later is more damaged than the former (Ashraf and Hussain, 1998; Ashraf et al., 2001a,b).

Among organic solutes, excess leakage of sugars may represent loss of respirable substrates from some seed species, whereas, others leak more amino acids than soluble sugars (Bewley and Black, 1986). Increased leakage of organic metabolites from deteriorated seeds might indirectly enhance their demise by encouraging the growth of contaminating microorganisms. It is possible that aging of seeds at relatively high moisture content brings about the hydrolysis of membrane components which cannot be replaced because of the insufficient activity of the appropriate repair processes. In addition, free radical formation might occur at both high and low moisture contents. The symptoms of low vigour and reduced viability could result from fundamental changes in membranes and macromolecules (Bewley and Black, 1986).

Loss in germinability and variations in E.C. and electrolytes in various cultivars of wheat seeds have already been documented in water-imibed-dried and salt-treated seeds (Ashraf and Hussain, 1998; Ashraf et al., 2000, 2001a, b) and present work is a continuation to understand these mechanisms in early hours of germination. The effect of gamma irradiation on the germination (Sattar et al., 1992) and growth and yield of barley has already been demonstrated (Subhan et al., 2004). The objectives of the present work are to demonstrate the effects of different doses of $\gamma$-radiation on the seed germination, E.C., changes in the efflux of inorganic and organic electrolytes, growth of seedlings and chlorophyll contents.
MATERIALS AND METHODS

Seed irradiation

Wheat seeds (cv. Inqalab) harvested in 1996 were irradiated by a gamma source (Cobalt-60) in Nuclear Institute for Food and Agriculture (NIFA), Peshawar. Treated seeds along with the un-irradiated control were stored at 25-30°C until used.

Electrical conductivity measurements

Ten wheat seeds (in duplicates) were immersed in 10 ml double distilled water (E.C.<10 μS/cm) and E.C. of leachates of imbibing seeds was measured at various time intervals with pre-calibrated conductivity meter (Milwaukee-CON1000). Data was expressed as specific conductance (μS/cm/seed) or in terms of rates of electrolytes leakage (μS/cm/seed/h).

Quantification of electrolytes in leachates

Among inorganic electrolytes, Na⁺ and K⁺ in leachates were measured after appropriate dilutions with flame photometer (Corning). Pi was measured by ammonium molybdate method. Briefly, 1ml leachate (40 seeds/10 ml water), was taken and 2 ml ammonium molybdate reagent (25 g ammonium molybdate pre-dissolved in 200 ml water was added to 400 ml water containing 280 ml conc. sulphuric acid. The mixture was cooled and filtered and volume made to 1L) was added and mixed. Then 2 ml stannous chloride reagent (0.5 g SnCl₂.2H₂O dissolved in 250 ml 2% HCl) was added and mixed and kept for 30 min. O.D. was measured at 700 nm (Plummer, 1990).

Amino acid contents and soluble proteins/peptides were determined by ninhydrin method (Plummer, 1990). Leachate (3 ml) was mixed with 2 ml ninhydrin reagent (ninhydrin, 1.6g; cadmium acetate, 4g; absolute ethanol, 80 ml; glacial acetic acid, 10 ml; distilled water, 20 ml). Contents were placed at 60°C for 20 min and absorbance was taken at 506 nm. For the determination of soluble sugars effluxed in leachates, 4 ml of 0.02% anthrone reagent in conc. H₂SO₄ (freshly made) was mixed with 1ml of leachate. The colour development after 20 minutes was measured at O.D. 620 nm against the blank reagent.

Seed germination

Wheat seeds (10 each in triplicates) were allowed to germinate in glass petri dishes containing double filter papers presoaked with distilled water. The petri dishes were placed at 20°C and germination was noticed until all the viable seeds had germinated for 3-days. Protrusion of radical was considered as an indication of the completion of germination.

Measurement of growth and chlorophyll contents

Once the active growth phase has started, number of secondary roots, root length and shoot length was monitored. Chlorophyll contents were measured by the method of Arnon (1949). The fresh leaves of the young seedlings were cut, weighed and homogenized in mortar and pestle in 80% acetone. The contents were filtered with miracloth and bench centrifuged for 5 min at 4000 rpm. The absorbance of the supernatant was measured and chlorophyll contents were calculated by the following formulae.

\[ \text{mg Chl a} = 9.93 \times \text{O.D.}_{660} - 0.777 \times \text{O.D.}_{643} \]
\[ \text{mg Chl b} = 17.60 \times \text{O.D.}_{660} - 2.81 \times \text{O.D.}_{643} \]

RESULTS AND DISCUSSION

Changes in electrical conductivity of leachates

The E.C. of un-irradiated controls and irradiated seeds was measured for the first 60 min of start of imbibition and results expressed in terms of μS/cm/seed (Fig.1). At zero time, E.C. of control seeds is 2.26 μS/cm/seed which is the lowest among all other irradiated seeds and there is 3.5 times increase during the next 10 min reaching 8.09
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However, for the irradiated seeds the lowest increase is 1.18 times (reaching 18.92 from 15.97 μS/cm/seed in 1Kr treated seeds) to 3 times increase in 50 Kr treated seeds (reaching 9.04 μS/cm/seed) after 10 min. As imbibition proceeds, little differences are observed among various treatments. When the data was expressed in terms of rates of E.C., μS/cm/seed/h, similar profiles were observed (Table 1). Highest rates of E.C. were observed in leachates of 1Kr treated seeds (57.2 ±15.76 μS/cm/seed/h) and lowest in untreated controls (25.04 ±6.55 μS/cm/seed/h). Similar profiles have been observed when wheat seeds were imbibed in water, varying sodium chloride concentrations and at various temperature regimes (Ashraf et al., 2000, 2001a,b). However, it is speculated that the biochemical and biophysical mechanisms operative during these stress conditions might be different (Bewley and Black, 1986).

Table 1. Rates of E.C. measurements (μS/cm/seed/h) of irradiated wheat seeds. (n=3). Only mean values are given here.

<table>
<thead>
<tr>
<th>Time</th>
<th>0Kr</th>
<th>0.5Kr</th>
<th>1Kr</th>
<th>5Kr</th>
<th>10Kr</th>
<th>20Kr</th>
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<td>-</td>
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<td>-</td>
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<tr>
<td>10</td>
<td>48.55</td>
<td>69.97</td>
<td>113.53</td>
<td>67.83</td>
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<td>58.55</td>
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<td>38.56</td>
<td>67.12</td>
<td>38.56</td>
<td>38.91</td>
<td>41.41</td>
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<td>30</td>
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<td>27.37</td>
<td>45.93</td>
<td>28.32</td>
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<td>29.51</td>
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<td>24.28</td>
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<td>21.96</td>
<td>35.16</td>
<td>22.85</td>
<td>21.96</td>
<td>23.56</td>
<td>17.49</td>
<td>19.28</td>
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<tr>
<td>60</td>
<td>11.42</td>
<td>15.47</td>
<td>24.28</td>
<td>16.07</td>
<td>15.35</td>
<td>16.42</td>
<td>12.38</td>
<td>13.57</td>
</tr>
<tr>
<td>mean</td>
<td>25.04 ±6.55</td>
<td>34.67 ±9.61</td>
<td>57.20 ±15.76</td>
<td>34.72 ±9.06</td>
<td>34.57 ±9.34</td>
<td>36.75 ±9.91</td>
<td>27.51 ±7.36</td>
<td>29.92 ±7.90</td>
</tr>
</tbody>
</table>

Table 2. Secondary root formation in wheat seeds. Number of secondary roots formed during the initial 5 -10 days of growing seedlings (n = 6-8). The numbers indicate minimum and maximum range of secondary roots.

<table>
<thead>
<tr>
<th>Day</th>
<th>0Kr</th>
<th>0.5Kr</th>
<th>1Kr</th>
<th>5Kr</th>
<th>10Kr</th>
<th>20Kr</th>
<th>50Kr</th>
<th>100Kr</th>
</tr>
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<td>2</td>
<td>2</td>
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<td>1-4</td>
<td>2-4</td>
<td>3-4</td>
<td>2-4</td>
<td>2</td>
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<td>3-4</td>
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<td>2</td>
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<td>3-6</td>
<td>3-5</td>
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<td>2</td>
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</tr>
</tbody>
</table>

Variations in efflux of inorganic electrolytes

A number of inorganic ions leake during seed imbibition. In the present studies Na+, K+ and Pi were measured in the leachates. Na+ contents found during day-1 and day-2 of imbibition remained ≤0.295 ppm/seed that increased to 0.625 ppm/seed on day-3 of imbibition. The differences in Na+ contents were similar between different doses of irradiated seeds (Fig. 2). Similar profiles were observed for K+ in the leachates. K+ were 0.844 ppm/seed in day-1 leachate, 1.072 ppm/seed in day-2 and 1.203 ppm/seed in day-3 old leachates (Fig. 3). The difference in efflux of K+ in leachates was insignificant between the control and irradiated seeds. These findings suggest that γ-irradiation has little or no effect on the leakage and rates of leakage of Na+ or K+ ions during early hours of imbibition of wheat seeds.
Fig. 1. E.C. measurements of irradiated wheat seeds (n=2) and s.e.m is ≤ 5%. Only mean values are given here.

Fig. 2. Determination of Na⁺ in leachates of 1-3 day imbibing wheat seeds (n=3), and s.e.m is ≤ 5%.

When leachates were employed for the estimation of inorganic phosphorus (Pi), a big increase was noticed on day-2 and day-3 old leachates (Fig. 4). Control seeds had 2.5 μg Pi/seed which increased 5 times in day-2 leachates and further increased to 40 μg/seed in day-3 leachates. Similar findings have been observed for the irradiated seeds. The main difference in irradiated seeds is that 12.5 μg/seed Pi is detected in day-1 leachate in 100Kr treatment that is maximum in all the treatments. However, in comparison with the untreated controls, 0.5 and 1Kr treatments had caused higher Pi leakage in solutes on day-3 compared with control or other treatments, though the values of Pi for all the treatments remained higher than that of control on all three leachates. These results suggest that the membrane becomes more susceptible to the loss of Pi after irradiations and such treated seeds on imbibition do not develop mechanisms to restrict Pi leakage in the surrounding medium for at least three days of imbibition.
Variations in efflux of organic electrolytes

Among the organic electrolytes effluxed during imbibition, only amino acids/proteins and water soluble sugars were estimated. Soluble sugar contents in leachates remained higher for un-irradiated seeds than irradiated ones for the day-1 of imbibition (Fig. 5). However, as the time to imbibition reached day-2 and day-3, soluble sugar contents increased more for the 20Kr and 10Kr treated seeds than that of control. Lowest sugar levels were seen in 1Kr and 100Kr treated seeds, i.e., 16.25 μg/seed. The sugar contents leaked during day-1 of imbibition may represent sugars present in the dry seeds, probably coming from free sugar pool or from sugars that are easily hydrolysed and on day-2 and day-3 old leachates, mobilization of reserved carbohydrates might have occurred.
Similarly, when amino acids and soluble protein contents in leachates were measured, un-irradiated seeds exhibited 72.5 nmoles/seed during day-1 of imbibition which were higher than any of the irradiated seeds except 0.5Kr treatment, where amino acid levels were 75 nmoles/seed (Fig. 6). Similar profiles were observed during the day-2 and day-3 of imbibition, reaching 102.5 nmoles/seed for control and 110 nmoles/seed for 0.5Kr treated seeds, whilst amino acid contents remained at low profiles in other treatments. These findings suggest that selective permeability of cellular membrane system might have been slightly modified that resulted in lower rates of amino acid leakage during the studied days of imbibition.

Fig. 5. Determination of soluble sugar contents in leachates of 1-3 day imbibing wheat seeds. The numbers 1-8 on x-axis code explained in Fig. 1.

Fig. 6. Determination of amino acid contents in leachates of imbibing wheat seeds. The numbers 1-8 on x-axis code explained in Fig. 1.
Germination profiles of irradiated seeds

Un-irradiated seeds have shown 93-97% germination (Fig. 7). However, all the irradiated seeds exhibited up to 60-90% germination; highest germination of 90% was observed by 5Kr and lowest (60%) by 100Kr treated seeds. As far as the rates of germination are concerned, germination completed in 35h in control and 60h in 100Kr seeds. These results demonstrate that the employment of irradiation to wheat seeds reduces their percent germination and
increases the spread of germination (Fig. 7). Ali (1997) has found reduced seed germination in $\gamma$-irradiated wheat seeds. Similar findings have been found in Capsicum annum seeds wherein 10-30 Kr doses reduced the seed germination (Khuspe and Ugale, 1977). Reduced germination due to higher doses of irradiation has been observed in Phaseolus aureus (Raghuvanshi et al., 1978), cotton (Ibrahim et al., 1981), sunflower (Iqbal et al., 1984) and Eucalyptus (Swarp and Kapoor, 1989).

Fig. 9. Shoot growth patterns (cm) of seedlings developed from the germination of irradiated wheat seeds (n = 6-8). The numbers 1-8 on x-axis code explained in Fig.1.

Fig. 10. Determination of chlorophyll contents of wheat seeds (n=3). The numbers 1-8 on x-axis code explained in Fig.1.
The increased spread of germination and reduced germination percentage has been attributed to several factors. For example, the synthesis of proteins is affected and overall biochemical changes produced due to higher doses of irradiation slowed down metabolism and did not cause its inhibition (Brezeanu et al., 1979). Total protein contents and non-protein nitrogen concentration has also been found less in irradiated seeds (Tikhonov et al., 1980). Degradation of protein reserves and proteinase activity has been reduced during germination of irradiated chickpea seeds (Khanna and Matherchandni, 1984). Much higher levels of acidic amino acids have been found in cotton seeds (Yuldashev et al., 1985). All these studies confirm the report of Brezeanu et al., (1979) that overall metabolism is reduced especially the protein metabolism which has been extensively studied and certainly this is the major factor affecting seed germinability.

Changes in seedling growth patterns and chlorophyll contents

The growth profiles of young seedlings was monitored from day 5-10, and number of secondary roots, shoot length, root length, and chlorophyll contents in young leaves (day-10) were measured (Table 2, Figs. 8-10).

Secondary roots formation was inversely proportional to the radiation doses (Table 2). Secondary roots did not develop in the irradiated (20-50 Kr) seeds and remained two whilst higher number of roots were noticed in control and other treatments. It seems irradiation has strongly affected those biochemical events that lead to the formation of secondary roots. When the length of roots was measured for the given time (Fig. 8), slow increase occurred in root length and rate of growth was lesser in seedlings belonging to higher doses of irradiation than the lower doses. Similar profiles were observed for shoot length (Fig. 9). These results clearly demonstrate that the metabolic processes are affected which result in slower growth rates and the effect may be mainly at chromosomal level. However, the induced growth patterns at 0.5-5Kr doses may be explained otherwise. In these cases, the irradiation might have induced the expression of those genes which were involved in rapid metabolism and genes of carbohydrate and protein metabolism might be the primary target. Whatever the mechanisms responsible may be, these changes are irreversible and un-repaired (Mukhtar, 1998).

Total chlorophyll contents (Fig. 10) recorded were highest in 1Kr (3.65 mg/ g leaf) treated seedlings followed by 0.5Kr and 5Kr (0.482 mg) treated seeds and the lowest contents were found in 100Kr (0.57 mg) treated seeds. When chl.a and chl.b were measured, chl.b contents were always 2-3 times higher in all the treated seedlings than chl.a contents. The ratio of chl.a/chl.b was maximal in 10Kr treated seedlings than that of others and minimum value was noticed in 100Kr treated seedlings. These findings suggest that the irradiation has affected those parts of the genome which are involved in the synthesis of chlorophyll. Dale et al., (1997) has determined chlorophyll contents in gamma-irradiated (0-450 Gy) potatoes and has found 50% reduction in chlorophyll contents and the response of different cultivars was different. It means some cultivars may be more resistant to irradiation treatment than the others. Increase of dose of gamma-irradiation in sunflower and soybean has been resulted in decrease in beta-carotene contents (Zeb and Ahmad, 2004).

In summary, the effect of γ-irradiation on seeds is multi-fold and depends upon the dose of radiation. Higher doses result in modifications in cellular membrane properties and lethal changes at genetic level which effect cellular differentiation processes resulting in altered rates of efflux of cellular metabolites and ions, reduced germination profiles and altered chlorophyll synthesis and root and shoot growth patterns (Bewley and Black, 1986). Work is continued to understand some of the biochemical mechanisms associated with these changes.

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