RADIATION EFFECT ON BIOSYNTHESES OF ASCORBIC ACID AND RIBOFLAVIN IN GERMINATING CHICKPEA

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ABSTRACT

The influence of irradiation on biosyntheses of ascorbic acid and riboflavin in germinating chickpea seeds at ambient (25– 35°C) conditions, was investigated. The rate of syntheses of these vitamins significantly increased with increasing germination time upto 120 h depending upon the treatment ($P<0.05$). Maximum amounts of ascorbic acid, 22.32 and 16.84 mg/100g, were found in the 0.10 kGy sample after 120 h of germination in tap and distilled water respectively. However, a radiation dose of 0.15 kGy resulted in the development of maximum values of riboflavin, 11.40 and 11.38μ g/g, on germination in tap and distilled water respectively. A significant linear relation ($r=0.954$ to 0.997) was observed between the biosyntheses of these vitamins and the germination time upto 120 h of irradiated and unirradiated chickpea seed ($P<0.05$).

Keywords: Irradiation Biosyntheses Ascorbic acid Riboflavin chickpea

I . INTRODUCTION

Legumes especially chickpeas occupy a prominent position among crops as they are the major protein component of diets on a worldwide basis. In Pakistan, chickpea is also used as animal feed in large quantities. Like other legumes, chickpea is deficient in some vitamins and the people who consume them as their staple food may develop specific deficiency diseases. Ascorbic acid is not generally detectable in grains. Biosyntheses of vitamins in seeds take place during plant growth or germination[1], which can be accelerated at low doses of gamma radiation[2]. Biosynthetic pathways for ascorbic acid and riboflavin in plants have been well established[3-5]. Germination is a physiological process, which involves complex macro and micro chemical components in biochemical environment of the seed. Gamma radiation either triggers or suppresses these biological reactions through direct action or indirectly as a consequence of radiolytic reaction products on hormones and enzymes[1]. Germination is an inexpensive and simple method of improving nutritive value, and several studies have reported higher levels of nutrients and lower values of antinutrients in germination food grains than ungerminated originals[6-9]. Levels of ascorbic acid and riboflavin in germinating corn at specific radiation doses, were found to be more than unirradiated controls[10]. The US, Food and Drug Administration (FDA) concluded in November, 1980 that food irradiated to doses upto 1.0 kGy is wholesome and safe for

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human consumption and needs no safety tests to market the irradiated food[11]. Sattar et al[12] recently observed that development of these vitamins was temperature dependent. This research was conducted to determined the influence of radiation and subsequent germination on the ascorbic acid and riboflavin contents of chickpea.

II. MATERIALS AND METHODS

Chickpeas (Cicer arientinum L.) of commercial blight resistant variety (CM– 72) were obtained from the Nuclear Agriculture Division of the Institute. The seeds were dried under sunlight to a moisture level of about 9%.

Germination The cleaned seeds were soaked in 4 – 5 volumes of water for 3 h. The water was then removed and batches of seeds were placed in plastic trays (30 × 45 cm) containing filter paper with embryo down. Germination was carried out for 24– 120 h at ambient room temperatures (20– 35°C) under the prevailing light and dark conditions during the day and night respectively.

Irradiation The seeds were irradiated with γ – ray at a dose of 0.05, 0.10, 0.15 and 0.20 kGy in a ISSLEDOVATEL (USSR) irradiator with a dose rate of 9.60 kGy/h.

Biochemical analysis The germinated seeds along with roots and shoots were taken every 3 h, dried at about 75°C in an air oven, ground in a Wiley mill to pass through a 40 mesh screen and stored in plastic bottles in a deep freezer for subsequent analyses. Moisture was determined by drying at 105°C. L. ascorbic acid was determined in the fresh samples whereas riboflavin in the oven dried materials. Vitamins assays were performed according to the methods of the Association of Vitamin Chemists[13]. Ascorbic acid was determined by titration with 2, 6 dichlorophenolindophenol and riboflavin by fluorimetry using a turner model 111 fluorometer with 2A and 47B as primary filters and 2A12 as a secondary filter.

Statistical analysis An estimate of relative variation was made by determining the coefficient of variation (*CV*) which is a ratio in percentage of standard deviation to the mean. Statistical significance of treatments was tested by the analysis of variance and the means were separated by the Duncan's multiple range test[14].

III. RESULT AND DISCUSSION

As ascorbic acid is important in fresh food materials, germinated fresh chickpea was analysed for this vitamin, and the influence of soaking and subsequent germination of irradiated (mean over 0.05– 0.20 kGy) and unirradiated seeds in tap and distilled water, is shown in Table 1. Ascorbic acid was not detectable in the seeds and the data revealed that the vitamin increased significantly throughout, reaching to levels of 16.55 and 15.98 mg/100g during germination of untreated seeds for 120 h in tap and distilled water respectively ($P < 0.05$). Mean ascorbic acid values over all the doses exhibited similar pattern. However, the influence of individual radiation dose in comparison with control significantly varied as presented in Figs.1– 2. It is evident that biosyntheses of ascorbic acid was greater in 0.1 kGy sample than others and that higher doses (0.15– 0.20 kGy) generally decreased synthesis when compared with unirradiated controls during germination in tap water (Fig.1). The maximum amount of the vitamin (22.32 mg/100g) was found in the 0.1 kGy sample after 120 h of

Table 1

Effect of gamma- irradiation and subsequent of germination on ascorbic acid content (mg/100g) of chickpea

Germination	Germination in tap water		Germination in distilled water	
	Unirradiated	Mean over	Unirradiated	Mean over
(h)		0.05 0.20 kGy		0.05– 0.20 kGy
Ungerminated	Undetectable	Undetectable	Undetectable	Undetectable
24	5.04^{a}	6.08^{a}	2.97^{a}	4.65^{a}
48	8.37^{b}	7.99^{b}	5.13^{b}	5.15^{b}
72	12.70^{c}	10.76^{c}	7.00^{c}	7.65^{c}
96	14.40^{d}	12.04^{d}	9.59^{d}	9.65^{d}
120	16.55^{e}	16.91^{e}	15.98^{e}	16.23^{e}
Mean	11.41	10.75	8.13	8.72
CV	41.82	40.09	61.67	54.01

a b c d e Each column values followed by different letters are significantly different ($P<0.05$)

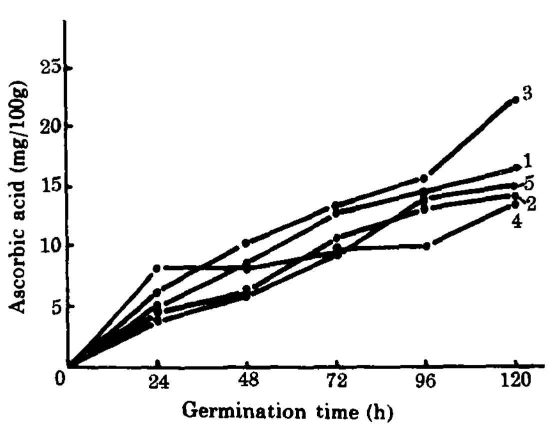

Fig. 1 Effect of irradiation and germination in tap- water on ascorbic acid

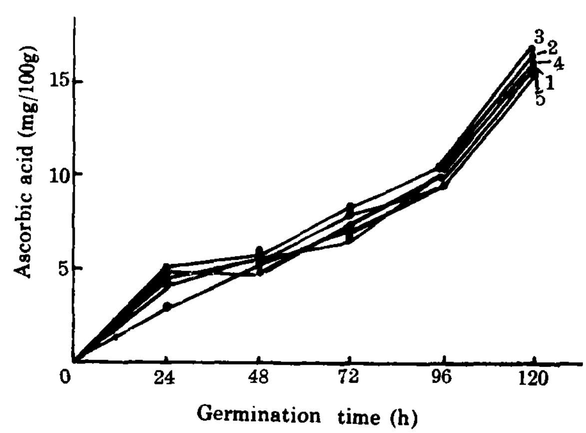

Fig.2 Effect of irradiation and germination in distilled water on ascorbic acid

1. Control 2. 0.05 kGy 3. 0.10 kGy 4. 0.15 kGy 5. 0.20 kGy

germination. The pattern of vitamin synthesis changed when germinaiton was carried out in distilled water. Although, again the maximum amount of ascorbic acid (16.84 mg/100g) was observed in 0.1 kGy sample, radiation treatment with all the doses generally exhibited slight acceleration in the synthesis as compared to control. Greater development of this vitamin on germination in tap than distilled water is obviously due to nutritional role of mineral elements during plant growth and development.

Statistical analysis of the data by the Analysis of Variance revealed significant influence of these variables on the ascorbic acid of chickpea ($P < 0.05$). The means were separated by the Duncan's multiple range test. In order to make an estimate of dispersion of the amount of vitamin in relation to germination, CV was measured. This revealed striking differences in the contents of ascorbic acid during germination. Determination of CV is especially appropriate under conditions where there are extreme values or when it is desired to express variation as a percentage of the average around which the deviations are taken. It was thought worth while to determine whether a significant regression of the amount in vitamin on germination period existed. The correlation coefficients, regression coefficients and regression equations of the data in question, were measured. The test statistics indicated the presence of a significant linear regression ($P < 0.05$). It was, therefore concluded that as the germination time was increased, the ascorbic acid increased and the relationship was expressed by significant linear regression of the forms:

$Y = 1.284 + 0.14x$ for control – tap water ($r = 0.985$)

$Y = 1.449 + 0.13x$ for 0.05– 0.20 kGy – tap– water ($r = 0.978$)

$Y = 0.465 + 0.12x$ for control– distilled water ($r = 0.976$)

$Y = 0.175 + 0.12x$ for 0.05– 0.20 kGy – distilled water ($r = 0.962$)

$Y = 0.611 + 0.13x$ for overall basis– tap and distilled water ($r = 0.954$)

Where x was germination time and Y was the amount of ascorbic acid. The amount of ascorbic acid was highly positively correlated with germination time of 120 h in each case. However, the significant linear relation between ascorbic acid and time of germination did not indicate overall zero– order reaction kinetics, hence the reaction can not be assumed to be unimolecular.

Although increases in ascorbic acid content in germinating seeds have been reported[15-17], kinetic of its development in relation to radiation treatment, has not been attempted except our own studies in corn. Distribution of ascorbate in vegetables and green leaves of several plants, has been tested and its metabolic activity was shown to be terribly influenced by light[4]. These studies suggest that low dose gamma radiation in addition to growth catalysing enzymes or hormones would have accelerated the metabolic activity of ascorbate during germination just like light radiations. Irradiation of tuber bulbs and grains at low doses with gamma or other ionizing rays is known to enhance sprouting. Germination (sprouting) involves slight growth of embryo (very young leaves) rather than to a stage of mature leaves. It has also been found that the ascorbic acid content has been found markedly higher in the younger leaves than mature leaves[4]. A dietary deficiency of ascorbic acid has been well known to cause scurvy in the human, guinea pig and the monkey; other mammals are capable of synthesizing their needs[5]. These studies will be of significant importance to people, who primarily live on cereals and legumes, which is the case in most of the developing and underdeveloped countries.

The effect of soaking and subsequent germination an riboflavin content of unirradiated and irradiated (mean over 0.05– 0.20 kGy) seeds is presented in Table 2. The vitamin values increased throughout from an initial amount of 1.66 μ g/g to maximum levels of 8.75 and 8.50 μ g/g in untreated chickpea on germination in tap and distilled water respectively. Mean riboflavin content over all the tested doses exhibited relatively higher values with a similar pattern. The riboflavin contents in relation to germination period in tap as well as distilled water for each irradiation

Table 2

Effect of γ – irradiation and subsequent germination on riboflavin content (μ g/g) of chickpea

Germination (h)	Germination in tap– water		Germination in distilled water	
	Unirradiated	Mean over 0.05– 0.20 kGy	Unirradiated	Mean over 0.05– 0.20 kGy
Ungerminated	1.66^{a}	1.65^{a}	1.66^{a}	1.65^{a}
24	3.50^{b}	3.80^{b}	2.50^{b}	3.35^{b}
48	4.16^{c}	5.70^{c}	4.00^{c}	4.73^{c}
72	5.00^{d}	6.96^{d}	5.00^{d}	6.15^{d}
96	8.02^{e}	9.26^{e}	7.50^{e}	7.85^{e}
120	8.75^{f}	11.51^{f}	9.50^{f}	10.00^{f}
Mean	5.19	6.47	5.02	5.91
CV	52.88	55.79	59.69	63.32

$^{a\,b\,c\,d\,e\,f}$ Each column values followed by different letters are significantly different ($P<0.05$)

dose and the unirradiated control are shown in Figs.3– 4. It was observed that biosyntheses of this vitamin was higher in irradiated than unirradiated seed and the values generally increased with increasing radiation dose during germination both in tap and distilled water. The maximum amounts of the vitamin, 11.40 and 11.38 μ g/g, were detected on germination of 0.15 kGy sample for 120 h in tap and distilled water respectively. Again statistical treatment of results indicated significant effect of irradiation and germination on the riboflavin values as well as presence of a significant linear regression ($P<0.05$) of the forms:

$Y = 1.621 + 0.06x$ for control– tap water ($r = 0.978$)

$Y = 1.699 + 0.08x$ for 0.05– 0.20 kGy– tap water ($r = 0.997$)

$Y = 1.084 + 0.07x$ for control– distilled water ($r = 0.985$)

$Y = 1.237 + 0.08x$ for 0.05– 0.20 kGy– distilled water ($r = 0.977$)

$Y = 1.410 + 0.07x$ for overall basis tap and distilled water ($r = 0.959$)

Where x was germination time and Y was the amount of riboflavin, which did not indicate overall zero– order reaction kinetics, hence the reaction could not be assumed to be unimolecular. The amount of riboflavin was found to be highly positively correlated with germination time up to 120 h.

Unlike ascorbic acid content, the reason for greater biosyntheses of riboflavin in distilled than tap water is not clear. An earlier study on irradiated corn indicated increases in this vitamin up to 96h of germination followed by decreases in each case; highest increase was found in 0.20 kGy sample followed by 0.15, 0.10 and control samples[10]. It has been recognized that riboflavin is formed from simple precursors in plants and certain microbes and that several plant extracts catalysed the conversion of 6,7 dimethyl- 8 ribilityllumazine to riboflavin and this reaction did not require any cofactor except the substrate[17]. Increase in the content of riboflavin and other vitamins during germination has been demonstrated by several workers[9-19]. Radiation induced acceleration of reactions involved in the biosyntheses of riboflavin provides reasonable basis for higher levels of this vitamin in treated than untreated seed in these studies. Recently it has shown that synthesis of vitamins such as ascorbic acid,

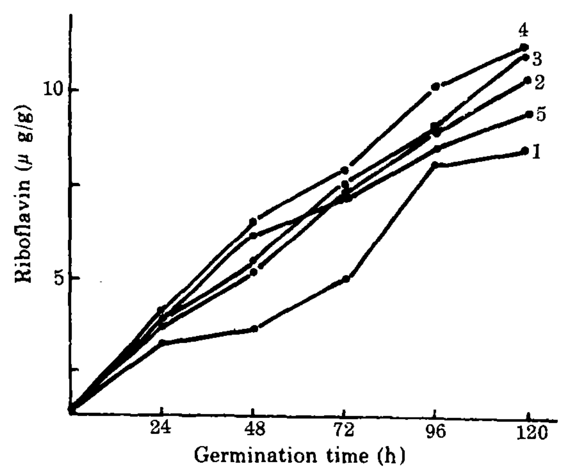

Fig. 3 Effect of irradiation and germination in tap- water on riboflavin

1. Control 2. 0.05 kGy 3. 0.10 kGy 4. 0.15 kGy 5. 0.20 kGy

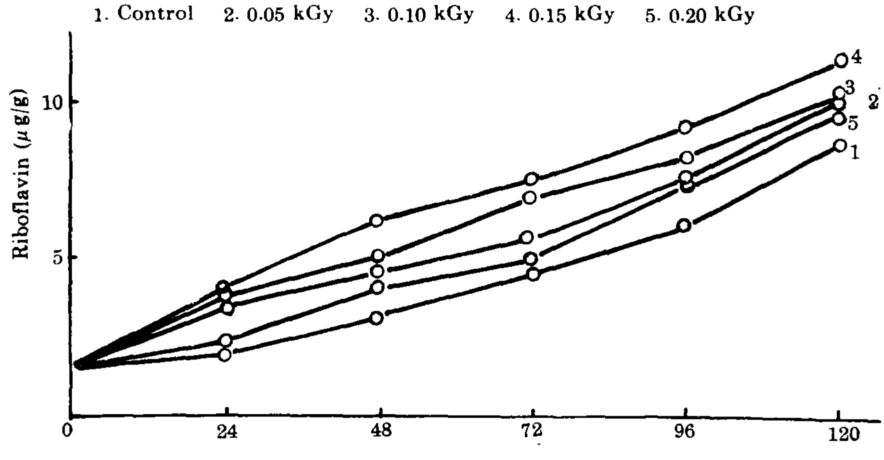

Fig. 4 Effect of irradiation and germination in distilled water on riboflavin

1. Control 2. 0.05 kGy 3. 0.10 kGy 4. 0.15 kGy 5. 0.20 kGy

riboflavin and thimain during germination is temperature dependent[12]. Hence it was concluded that low doses of γ – radiations stimulated biosyntheses of ascorbic acid and riboflavin in germinating chickpea. A significant linear relation was observed between the rate of biosyntheses and germination time up to 120 h.

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