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Influence of germination techniques on sprout yield, biosynthesis of ascorbic acid and cooking ability, in chickpea (*Cicer arietinum* L.)

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Abstract

Effects of germination time and illuminations on sprout yield, biosynthesis of ascorbic acid, cooking ability and moisture accumulation in chickpeas were significant ($p \leqslant 0.01$). Green light had the highest promoting effect on the ascorbic acid level (40.59 mg/100 g) as compared to other illuminations but significantly reduced the sprout yield (188.6 g) as compared to dark, fluorescence and γ-rays illuminations with significantly high sprout yield (196 g) and imbibing moisture (51%). Cooking time was reduced by 43% due to γ-rays in un-soaked seed. Cooking time increased in all treated chickpea samples after 24 h germination and thereafter decreased significantly. Red light significantly increased the cooking time (68.44 min) followed by fluorescent (64.5 min), yellow (61.8 min) and green light (60.9 min). The results indicated that germination of chickpea under green light was an effective process in enhancing ascorbic acid content while dark, fluorescence and γ-rays were effective in promoting sprout growth and to some extent biosynthesis of ascorbic acid.

Keywords: Sprout yield; Ascorbic acid; Cooking time; Moisture level

1. Introduction

Chickpea (*Cicer arietinum* L.) is an ancient crop and has been grown and consumed in tropical, sub-tropical and temperate regions for centuries. It is valued for its nutritive seeds with high protein content, 17–22% and 25.3–28.9%, before and after dehulling, respectively. Chickpea is used exclusively as food in many countries, though it is also used as livestock feed in Mexico (Malhotra, Pundir, & Slinkard, 1987; Muehlbauer & Singh, 1987). Traditional uses include boiling, roasting, canning or processing into hummus (a traditional dish in the Middle East).

Sprouting is the practice of soaking, draining and leaving seeds until they germinate and begin to sprout. It has been identified as an inexpensive and effective technology for improving the nutritional quality of cereals and grain legumes. As water is introduced, enzyme inhibitors are disabled and the seed explodes to life (Bau, Villaume, Nicolas, & Mejean, 1997; Chang & Harrold, 1988; Frias, Diaz-Pollan, Hedley, & Vidal-Valverde, 1995; Reddy, Sathe, & Salunkhe, 1989; Schhulze et al., 1997). Germination unfolds, and enzymes trigger elaborate biochemical changes. Proteins break into amino acids. Water-soluble vitamins such as B complex and vitamin C are created (Sattar, Badshah, & Aurang, 1995 and Zielinski, Frias, Mariusz, Kozlowska, & Vidal-Valverde, 2005). Fats and carbohydrates are converted into simple sugars. Weight increases as the seed absorbs water and minerals. Dry pulses are considered to be a poor source of vitamin C (Mao-Jun, Dong, & Mu-Yuan, 2005). Sprouting induces biosynthesis of this vitamin considerably (Sattar et al., 1995). Thiamin and Niacin are readily available through sprouts. Vitamin A content of

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seeds is improved considerably after sprouting. Chickpea sprouts are good sources of folic acid. Proteins from sprouts are considered to be good alternates to the costly animal proteins. Among food legumes, chickpea is the most hypocholesteremic agent; germinated chickpea was reported to be effective in controlling cholesterol level in rats (Geervani, 1991). In addition to this, consumer's acceptability (cook ability, taste and flavor) is also increased through sprouting (Badshah, Gul, Zahid, Bibi, & Ihsanullah, 2006, 2003).

Since the quality and quantity of bioactive compounds are important when the sprouts are considered as a new functional food, the present study was undertaken to investigate the effect of germination techniques on sprout yield, biosynthesis of ascorbic acid, cook ability and their relationship in chickpea.

2. Materials and methods

The experimental work was carried out in Food Science Division of the Nuclear Institute for Food and Agriculture (NIFA) Peshawar. Seeds of desi type Chickpea Variety, NIFA-2005, evolved at NIFA, were processed for sprouting as per following procedure:

2.1. Preparation of samples for sprouting

The chickpea seeds were cleaned from all impurities including broken and diseased seeds. Part of the un-soaked sample was ground in stainless steel grinder to pass through a standard 40 mesh screen. The ground samples were kept in plastic bags, stored at 4 °C for chemical determinations.

2.1.1. Soaking of chickpea seeds

The seeds were soaked by submerging in tap water in glass containers for 24 h at room temperature. After pouring off the soaking water, the seeds were rinsed with water, spread evenly on a tray lined with absorbent paper and then placed in a controlled environment chamber at 28 °C.

2.1.2. Sprouting chamber

Wooden chambers each with $91 \times 91 \times 60$ cm ($L \times H \times W$) dimensions were used for germination of seeds. There were five chambers used for five types of illuminations, i.e., fluorescence, yellow, blue, green and red and two for dark and gamma irradiated samples. The light source in the illuminated chambers was fitted on the ceiling of the chamber. The temperature of the chambers was maintained at 28 ± 3 °C.

2.1.3. Gamma irradiation treatment

Seeds were irradiated at a dose of 3 krad in the Gamma Research Irradiator (Isseldovatel, USSR) available at this Institute. Soaking and sprouting was then carried out in dark conditions.

2.1.4. Sprouting procedure

Sprouting was started in triplicate for each treatment (illumination i.e., dark, red, blue, tungsten, green and fluorescence and length of time i.e., 0, 24, 48, 72, and 96 h) in trays lined with absorbent (filter parer sheets) paper. Seed/sprouts were washed twice a day to avoid microbial growth. Tape water was sprayed through out germination periods at 9 a.m., 1 p.m. and 6 p.m. daily.

2.1.5. Light exposure

Fluorescent tubes (40 W, Philips, Lahore, Pakistan) were used as white light source. Respective colored bulb (40 W, Philips, Lahore, Pakistan) were used as per illumination treatments. The trays were distributed under the light so as to give uniform flux density to each tray. The same flux density were obtained by turning on the fixed number of light sources and by adjusting fixed distances between the lamps and the test materials.

2.2. Assay of physicochemical characteristics

2.2.1. Ascorbic acid

Fresh samples from each treatment were taken for ascorbic acid estimation. The direct colorimetric method was used for the measurement of this vitamin which is based on the measurement of the extent to which a 2,-dichlorophenol–indophenol solution is decolorized by ascorbic acid in sample extracts and in standard ascorbic acid solutions (AOAC, 1984 method # 43.064). Since interfering substances reduce the dye slowly, rapid determination would be measuring mainly the ascorbic acid.

2.2.2. Cooking time

Cooking time was determined by placing 25 g of seed in boiling water. Cooking was complete when color of 80% to 100% of seed (cotyledon) changed completely from whitish yellow to yellow due to gelatinization (Badshah et al., 2006, 2003).Cooking time was checked after 60 min boiling by taking out some seeds for observing the color and continued at 5 min interval.

2.3. Statistical analysis

Statistical analysis was conducted for each of the measured traits by analysis of variance (ANOVA- using CRD factorial design) and the means were separated by Duncan Multiple Range test (DMR) using Mstat-C software (SAS, 1996). The relationship between sprout yield (g) as well as cooking time to germination time was fitted to a polynomial regression equation of the form:

$$Y = ax^3 + bx^2 + cx + d$$

where

Y = sprout yield (g) or cooking time (min)
X = germination time (h),

a, b and c are the coefficients of the third degree polynomial equation

While that of ascorbic acid to germination time was fitted to regression analysis:

$$Y = a + bx$$

where

Y = ascorbic acid
a = intercept
b = slope

3. Results and discussion

3.1. Sprout yield

Growth of sprout (yield) was significantly influenced ($p < 0.01$) by germination time, type of illumination and their interaction (Fig. 1, Table 1). As a result of illumination effect, maximum mean values were noted for dark, fluorescence and γ-rays sprouts followed by yellow and red and minimum for green and blue. The sprout yield increased as the germination time increased Table 2 and the highest value was noted with 120 h of germination time (214.3 g). Highest sprout yield (228.4) in γ-rays irradiated seeds was obtained after 120 h of germination, while in un-irradiated seeds, germinated in dark, the maximum was reached after 96 h. Fluorescent light, which also had pronounced effect on sprout growth of chickpea, gave highest yield after 96 h germination (224.2 g). Green and blue lights significantly inhibited sprout growth ($p < 0.01$) and maximum value noted for these lights were 212.8 g after 96 h and 213.6 g after 72 h, respectively. A slightly lower inhibitory effect on sprout growth was exhibited by yellow and red light. For yellow light, sprout yield decreased from the 24 h soaking value of 208.7–203.0 g after 96 h germination but then increased to 222.1 g after 120 h germination.

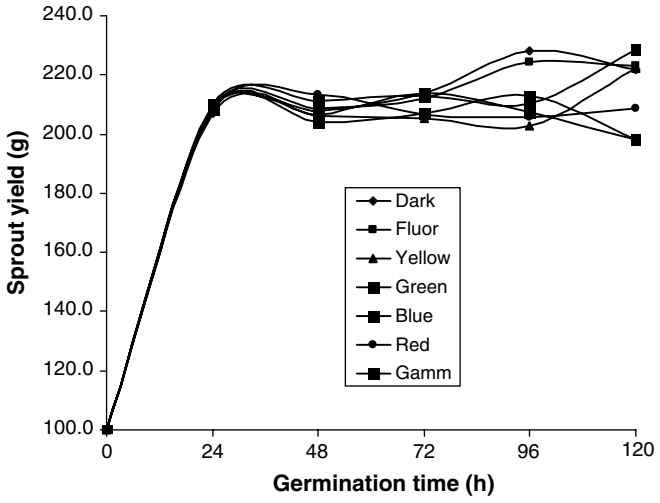

Fig. 1. Effect of germination time and illumination on sprout yield of chickpea.

3.2. Ascorbic acid

The biosynthesis of ascorbic acid in plants is highly responsive to a wide variety of environmental factors (Davey et al., 2000). Several groups have reported that plants increase their ascorbic acid levels in response to different light intensity (Foyer, Lelandais, Edwards, & Mullineaux, 1991; Logan, Barker, Demmig-Adams, & Adama, 1996), but they did not provide information about the effects of different light types on biosynthesis of ascorbic acid. The effect of germination time and type of illuminations is depicted in Fig. 2 and Table 1. The results showed that green light significantly ($p < 0.01$) increased the promotion of biosynthesis of ascorbic acid in chickpea sprouts but inhabited their growth (Fig. 1). Linear relationship was noted (Fig. 2, Table 3) between ascorbic acid and germination time. The increase in ascorbic acid with the advancement of germination time is well documented (Mao-Jun et al., 2005). The interaction of germination time and type of illumination was also highly significant ($p < 0.01$). The highest values for dark, fluorescent, yellow, green, blue, red lights and γ-rays were 60.4, 60.1, 61.6, 63.3, 59.7, 59.0 and 61.4 mg/100 g, respectively, after 120 (in case of dark, fluorescence, yellow, green and blue light) and 96 h (in case of red light and γ-rays). With γ-rays biosynthesis of ascorbic acid was very slow during the first 24 and 48 h of germination, while it increased tremendously after 72 and 96 h of germination. This might be attributed to some biochemical changes in seed embryo which might be restored/rearranged thereafter.

Ascorbic acid has many important biological functions in both animals and plants (Ghosh, Mukhopadhyay, & Chatterjee, 1997; Ginter, 1989). It was reported by Mao-Jun et al. (2005) that germination process, in particular under ultraviolet illumination has a significant promoting effect on the ascorbic acid level in soybean and inhibiting effect on sprout growth. They observed red light to be effective in enhancing sprout yield while in our study dark, fluorescence and γ-rays were more effective in this regard. Our results are well in agreement with those of Fernandez and Berry (1988) who reported a significant increase in ascorbic acid during chickpea germination. It was also reported by Riddoch, Mills, and Duthie (1998) that many species of pulses produced significant quantities of vitamin C up to five days germination in both light and dark, while cooking caused a marked decrease in this vitamin. Yang, Basu, and Ooraikul (2001) reported that the concentrations of ascorbic acid, vitamin E and beta-carotene steadily increased in wheat with increasing germination time, reaching their peaks after 7 days. Significant increase in the content of ascorbic acid of different cereals and legumes seeds has also been reported by Harmuth-Hoene, Bognar, Kornemann, and Diehl (1987).

Table 1
Analysis of variance showing mean sums of the squares for all parameters

Source of variation	Degrees of freedom	Ascorbic acid (mg/100 g)	Sprout yield (g)	Cooking time (min)	Moisture content (%)
Illumination	6	9.74***	217.19***	385.37***	6.60***
Sprouting time	5	804.79***	43109.95***	10177.00***	8504.21***
Illumination X Sprouting time	30	4.61***	121.16***	316.42***	2.47***
Error	84	0.89	1.46	24.72	0.40
Total	125				

*** $P < 0.001$ (Highly significant).

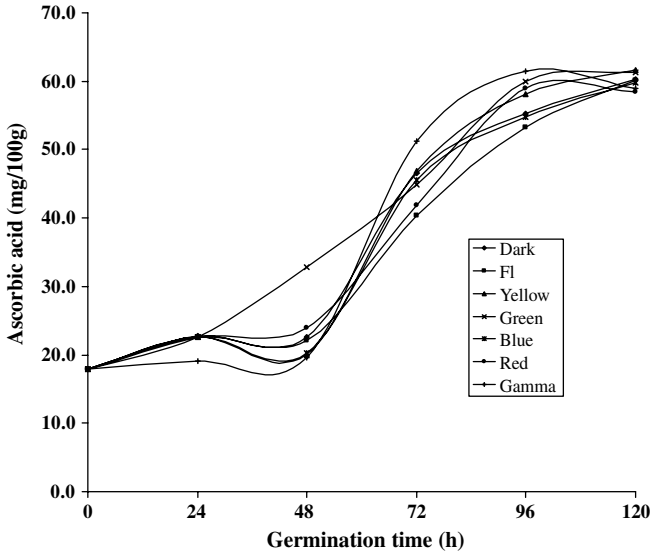

Fig. 2. Effect of germination time and illumination on ascorbic acid content of chickpea.

Table 2
Polynomial relationships between germination time and sprout yield in chickpea

Illuminations	*a*	*b*	*c*	*d*	R^2
Dark	0.0003	0.0684	5.0147	105.47	0.94
Fluorescence	0.0003	0.0751	5.2676	105.21	0.94
Yellow	0.0004	0.0927	5.8269	103.8	0.96
Green	0.0003	0.0722	5.1246	105.63	0.92
Blue	0.0003	0.0751	5.3098	104.6	0.94
Red	0.0004	0.0878	5.7802	103.63	0.97
γ-rays	0.0004	0.0909	5.8704	103.72	0.97

Table 3
Linear relationships between germination time and ascorbic acid in chickpea

Illuminations	*a*	*b*	R^2
Dark	13.739	0.3971	0.91
Fluorescence	13.101	0.3822	0.92
Yellow	12.709	0.4193	0.88
Green	15.521	0.4058	0.97
Blue	13.13	0.3946	0.89
Red	13.7	0.3929	0.91
γ-rays	11.995	0.4337	0.84

3.3. Cooking time

The influence of sprouting time and illuminations and their interaction on cooking time was highly significant (Fig. 3, Tables 1 and 4). The cooking time first increased from 70 to 100 min (40–90 in case of γ-rays) after 24 h soaking/germination and thereafter it decreased significantly in almost all illumination treatments. As a result of illumination effect, the lowest time for cooking was observed with γ-rays (55 min) samples followed by blue light sprout and maximum time for cooking (68 min) was noted with red light illumination followed by fluorescent light (64.5 min).

As can be noted the gamma irradiation treated unsprouted seeds had drastically reduced (43%) cooking time as compared to the untreated seeds. Reduction in cooking time of legumes due to irradiation has previously been observed in our earlier studies. It was found in those stud-

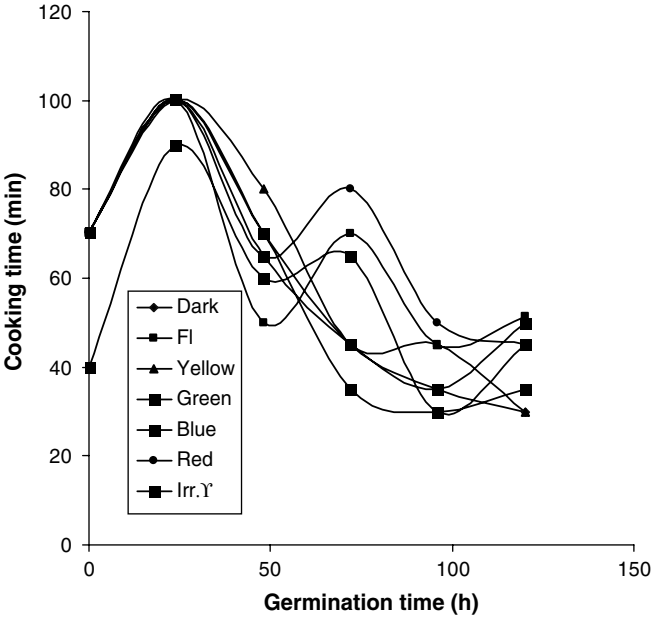

Fig. 3. Effect of germination time and illumination on cooking time of chickpea.

Table 4
Polynomial relationships between germination time and cooking time in chickpea

Illuminations	*a*	*b*	*c*	*d*	R^2
Dark	0.0002	0.0458	1.7252	73.18	0.94
Fluorescence	0.0001	0.0261	0.8986	74.80	0.51
Yellow	0.0002	0.0437	1.7764	72.94	0.91
Green	0.0003	0.0517	1.8266	73.101	0.93
Blue	0.0003	0.0557	1.9933	72.90	0.95
Red	0.0001	0.0247	1.1346	73.458	0.69
γ-rays	0.0003	0.0573	2.7932	42.302	0.77

Table 5
Effect of germination time and illuminations on moisture content of chickpea sprout

Germination time (h)	Illuminations							
	Dark	Fl	Yellow	Green	Blue	Red	γ-rays	Mean
0	9.7k	9.7k	9.7k	9.7k	9.7k	9.7k	9.7k	**9.7f**
24	57.7ghij	57.7ghij	57.7ghij	58.4ghij	57.7ghij	57.7ghij	57.7ghij	**57.8d**
48	57.1hij	56.3ij	57.2hij	57.2hij	57.8ghij	56.2j	58.7fgh	**57.2e**
72	59.0efg	58.7fgh	57.4ghij	57.9gh	58.7fgh	57.8ghij	60.8bcd	**58.6c**
96	62.00ab	61.6abc	58.5fgh	58.7fgh	57.9ghi	59.0efg	60.6bcd	**59.8b**
120	62.57a	62.4a	61.61abc	60.3cde	59.9def	59.5def	61.7abc	**61.1a**
Mean	**51.4a**	**51.1a**	**50.4b**	**50.4b**	**50.3b**	**50.0b**	**51.6a**	

Values are averages of three determinations per replication, with three replications per treatment.
Values in the interaction matrix followed by different letters are significantly ($P < 0.01$) different from each other.
Mean values for illuminations (row) or germination time (column) followed by different letters are significantly ($P < 0.01$) different from each other.

ies that most of the reduction in cooking time was achieved with lower irradiation dose (1 kG y), while further reduction with higher doses was only nominal. It was further noted during those studies that cooking time reduction was more pronounced in chickpea than other legumes (Zeb, Ahmed, Shah, & Bibi, 1990, 1991). Since the sprouted samples were oven dried before cooking time determination, the 24 h sprouted samples for all illuminations showed an increase in the cooking time, however, longer sprouting time resulted in significantly ($p < 0.01$) shorter cooking time.

Evidence regarding the influence of germination time and conditions thereof is lacking. It has previously been postulated that cooking time among other factors, is influenced by the phytic acid contents of the seed as reported by Zeb, Bibi, Shah, and Sattar (1991). Since this compound is hydrolyzed during spouting process (Camacho, Sierra, Campos, Guzman, & Marcus, 1992), the reduction in seed cooking time after 48 h sprouting could have been the result of that chemical change. According to Khaleque, Elias, Braham, and Bressani (1985), biochemical changes like enhancement in nitrogen solubility due to germination could also be related to the changes in cooking time. The initial increase in the cooking time (24 h germination time) could be due to the early seizure of these unfolding biochemical processes by oven drying.

3.4. Moisture content

Moisture level of sprouts was significantly influenced by germination time. The mean moisture content of the samples increased markedly from 9.7 to 61.1 with increase in germination time from 0 to 120 h. The illuminations effect was also statistically significant ($p < 0.01$). Dark, fluorescence and γ-rays sprouts had the maximum levels of moisture (51.1) as compared to yellow and green (50.4), blue (50.3) and red illuminations (Table 5).

4. Conclusions

This study has demonstrated that the germination process and illumination type, particularly green illumination had significant promoting effects on the ascorbic acid content of chickpea. Germination under dark, fluorescence and γ-rays appears to be effective in increasing the sprout yield as well as enhancing quality of chickpea sprouts.

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