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Full Length Article

Improved Growth and Metabolism of Sunflower *via* Physical Seed Pretreatments

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Abstract

Physical pre-sowing seed treatments as low-cost ecofriendly strategy for better growth and yield of food crops are well accredited. This study was conducted to find out the possible role of physical pre-sowing seed treatments to improve growth and metabolism of sunflower. Seeds were subjected to pre-sowing gamma (γ) irradiation (0, 10, 20 and 30 kGy), He-Ne laser (0, 1, 2, 3 min), UV-B (0, 20, 30, 40 min) and magnetic field (0. 0.1 Tesla, 0.2 Tesla, 0.3 Tesla) treatments. At vegetative and flowering stages, photosynthetic pigments, carotenoids, proteins, flavonoids, soluble sugars, reducing sugars, total soluble phenolics and anthocyanins were evaluated. Results indicated that γ-radiation (10 kGy) and He-Ne (at 1 and 2 min) increased leaf biomass. Along with gamma and UV treatments, He-Ne laser and magnetic field treatment also significantly enhanced the achenes per capitulum and seed oil contents. Chlorophyll contents were higher at vegetative stage as compared to flowering stage. Total soluble proteins, total soluble sugars and total phenolics were increased in all treatments at their all levels. Moreover, flavonoid contents were increased by all pre-sowing, but reverse was displayed by anthocyanin at vegetative stage and no effect was noted at flowering stage. However, oil contents showed increase only in He-Ne or magnetic pretreatment and decreased in response to gamma and UV radiation treatment. In conclusion, physical seed pretreatments proved pragmatic option to improve growth and metabolite accumulation especially at vegetative stage in sunflower. Moreover, lower doses of gamma, UV and He-Ne laser and all the doses of magnetic treatment improved the yield in term of achenes per capitulum along with achene oil percentage.

Keywords: Magnetic field; UV radiations; γ-radiation; He-Ne laser; Metabolites

Introduction

Expansion in agricultural production is global need of time due to uncontrolled population growth (Asghar *et al.*, 2016). Efforts have been directed to maximize the yield by modifying the agronomic practices. One of the most target-oriented approaches in enhancing plant productivity is to create plants with desired traits by employing successive assortment through plant breeding (Moose and Mumm, 2008). However classical breeding approaches offer labor and time barriers (Collard and Mackill, 2008). Thus, alternative strategies with rational cost, time and labor are direly needed.

Mutation based molecular breeding, by using different mutagenic agents and molecular tools offer attractive and comprehensive choice to get plants with desired traits (Majeed *et al*., 2017). Compared to other molecular approaches, use of mutagenic agents such as different irradiation has been observed to be quick and easy to practice (Çelik and Atak, 2017). Moreover, such physical approaches are being employed in the economically competitive context to search economic strategies.

Seed physical treatments have been observed to be the source of energy which is absorbed by the different molecules present in the cells. This imported and then transformed energy stimulates the enzymes, accelerating the seed metabolism, early germination and stimulation of plant development (Stutte *et al*., 2009; Hernandez *et al*., 2010; Velijević *et al.*, 2018). Unlike chemical application, where the necessary substances are directly inserted into the cell, when seeds are subjected to physical treatments, energy introduced in the cell results in conditions for molecular transformations either at genetic as well as at metabolic level (Govindaraj *et al*., 2017).

Seed physical treatments have become practicable choice for researchers to get desired metabolic attributes (Macovei *et al.*, 2014). As all the metabolic traits are under the influence of genetic makeup of the cell, hence any positive modification in this architecture may help to modulate metabolic activities. Thus, exploration of such agents, which may contribute in positive alteration of genetic and ultimately the metabolic activities, is of great significance. The mutagenic agents, which have already been explored include the magnetic (Bukhari *et al.*, 2019),

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He-Ne laser (Arafa *et al*., 2018), UV irradiation, gamma elicitations and ultrasounds (Le *et al.,* 2019). However, the effective dose of above-mentioned agents is specific depending on plant species and exposure time **(**Marcu *et al*., 2013a). Thus, for each crop systemized optimization of doses is of much more importance in order to get better primary and secondary metabolite fluxes. Therefore, it was hypothesized that physical seed pretreatments may modulate the metabolites flux by producing changes in the secondary metabolic pathways. The current study is first of its kind focusing on the dose optimization by using more than one sources of physical treatment with different physical seed pretreatments for the comparison of primary and secondary metabolites in sunflower (*Helianthus annuus* L.) at different growth stages.

Materials and Methods

Seed Source and Selection of Levels

The experiment was conducted in experimental field of Govt. College University Faisalabad, New Campus. Seeds of FH-129 variety were obtained from Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan. Seeds were subjected to the pre-sowing treatments with water (12 h), magnetic field (0, 0.1, 0.2 and 0.3 Tesla for 20 min), ultraviolet (UV-B) doses (for 0, 20, 40 and 60 min), He-Ne laser treatment (for 0, 1, 2 and 3 min) and gamma (γ) irradiation (with 0, 10, 20 and 30 kGy). Sowing was done in field (plot size of 24 × 5 meter) using randomized complete bock design (RCBD). Row to row and plant to plant spacing was maintained as 60 cm and 20 cm, respectively. Soil was fertigated with full dose of recommended chemical fertilizers (150-60-60 NPK kg ha^{-1}). Nitrogen fertilizer was used in different splits at different critical stages of crop. One-third dose of nitrogen as urea was mixed in soil at planting time. While the remaining two-thirds of nitrogen were splitted into two doses; one was applied at vegetative stage (20 days after sowing) and the second at the flowering stage of the crop. The phosphorus (P) and potassium (K) were applied as Triple Super Phosphate and Sulphate of potash (K_2SO_4) at the time of seed bed preparation. All cultural practices such as irrigation, weed management, hoeing and plant protection *etc*. were kept normal for the crop during the experiment.

First harvest was taken at vegetative stage when the plants were three-week-old. For second harvest data was collected at flowering stage (60 days old plant). The biochemical aspects were studied at both vegetative and flowering stages while the growth attributes were recorded only at flowering stage. Final harvest was taken when back of the heads turned yellow and bracts were brown and dried for 4 to 5 days. Five capitulums were selected for the determination of different yield components. After measuring the cake weight, grains were separated. No. of Achenes per cake were counted and 100 Achenes weight was measured. The individual parameters were quantified by averaging the five readings.

Leaf Analysis for Metabolites Accumulation

Photosynthetic pigments: The chlorophyll a and b were estimated following Arnon (1949) while the carotenoids were determined with method of Davis (1976). Absorbance of the leaf extracted with 80% acetone was recorded at 645, 663 nm and 480 nm.

Total phenolics, flavonoids and total protein contents: The total phenolics were conducted as described by Julkenen-Tiitto (1985) using Folin Ciocalteu's reagent. Sample extract was prepared as prescribed in procedure. After 90 min of incubation, the absorbance was measured at 765 nm using spectrophotometer. Total flavonoid contents were measured by following detail procedure of Marinova *et al*. (2005) while total protein contents were determined by the method described by Bradford (1976). Reaction mixture was prepared and absorbance of the sample was determined by spectrophotometer at 595 nm.

Reducing sugars contents and total soluble sugars: Reducing sugars contents were estimated as described by Miller (1972). Reaction mixture was vortexed and absorbance was taken at 540 nm. Total soluble sugars were determined with the method of Yoshida *et al.* (1976). After vortexing, the reaction mixture was heated at 95°C for 15 min. Absorbance of samples was measured at 625 nm.

Total Anthocyanin content: The quantitative analysis of anthocyanin was performed spectrophotometrically by the method of Nozzolillo (1978). Acidified methanolic leaf extract (3 mL) was taken and absorbance was recorded by using spectrophotometer at 536 and 600 nm.

Determination of oil content: Samples were made oil free for the estimation of percent oil content by following A.O.A.C. (1996). Three g plant sample (leaves/grains) was taken in plastic tube (50 mL capacity) and 30 mL n-hexane was added to it. Tubes were placed on shaker for 24 h at 100 rpm. Afterwards, samples were centrifuged at 3500 g for 18 min at 20°C and supernatant collected. Two successive repetitions of extractions were made on the residue by vortexing and centrifugation. Pooled but cleared extracts of all three collections were preserved for oil estimation. Whereas residue was dried as fat free sample, difference of weight after extraction was recorded as fat content.

Growth and Yield

Plant growth in terms of leaf fresh weight and dry weight and yield attributes like cake weight and achenes weight were determined in gram via electronic balance. Achenes per cake were counted and noted. For growth and yield five plants were used for single replicate.

Statistical Analysis

Data collected was subjected to Costat CoHrot 6.4 software. Analysis of variance technique (ANOVA) and least significant difference (LSD) test were used to compare the significance ($P < 0.05$) of variance sources and comparison of treatments means.

Results

Plant Growth and Yield

Growth of plants in terms of leaf biomass was variably affected by applications; however, this effect was treatment type and treatment level specific (Table 1). An increasing trend in both fresh and dry weight was observed in plants raised from seeds treated with 10 kGy gamma and 2 and 3 min of He-Ne radiation. In case of UV, only 60 min treatment level showed significant increase in leaf fresh weight but not in leaf dry weight (Table 1). Number of achenes per cake underwent significant increase in response to10 kGy and 20 kGy gamma radiation treatments while decreasing effect was recorded at 30kGy of gamma treatment radiation level. However, in case of He-Ne only 2 min treatment level while 40 and 60 min treatment levels of UV significantly increased the number of achenes per cake and were found equally effective. Moreover, all the three levels of magnetic treatment (0.1, 0.2 and 0.3 Tesla for 20 min) showed significant improvement in number of achenes per cake (Table 1).

In all the treatments a significant decrease in cake weight was found except all the levels of magnetic treatment and 10 kGy of gamma radiation where this decrease was non-significant as well as in 20 kGy treatment of gamma radiation where non-significant increase was observed. Similarly, a significant decrease was observed in 100 achene weight in all the treatments except He-Ne radiation where this yield attribute underwent non-significant change. This decrease in 100 achene weight in plants grown from seeds treated with gamma radiation was significant at 20 kGy treatment level only as compared to non-treated or hydro-primed treatments whereas in UV only 3 min treatment level and in magnetic treatments only 0.3 Tesla showed significantly decreased values.

In case of percentage seed oil contents all types of treatments were found significantly effective in increasing seed oil contents except UV treatment where non-significant increase was observed. However, this increase was observed to be treatment level specific. In case of gamma 10 kGy and 30 kGy and in case of magnetic treatment 0.1 T and 0.3 T showed significant increase. Moreover, such significant increase was also found only in seeds treated He-Ne for 2 min. The maximum increase was observed in 0.2 T treatment level of magnetic treatment followed by 30 kGy treatment level of gamma irradiation.

Plant Pigmentation

Leaf pigmentation was also affected significantly due to different physical seed treatments, but the effect was treatment type and level specific (Fig. 1). An increase in chlorophyll a was found due to all treatments except to that of gamma and 1 min He-Ne radiation treatment (Fig. 1A–B). Similarly, at flowering stage although non-significant but an increase in leaf chlorophyll a was recorded due to all treatments except to that of magnetic treatment where the reverse was true at all the levels. Only 10 kGy of both gamma and 20 min UV treatment showed such non-significant increase compared to plants grown from treated or hydro primed seeds. All the three levels of He-Ne treatment to seed showed non-significant increase.

At vegetative stage an increase in leaf Chlorophyll b was found except to that of lower levels of gamma (10 kGy and 20 kGy) and the He-Ne radiation (1 and 2 min) where a decreasing trend in chlorophyll b was found. This increase was significant in 30 kGy of gamma and 3 min He-Ne treatment only. In all the levels of UV and Magnetic treatment non-significant increase was noted. Similarly, at the flowering stage a clear decrease was recorded at 0.1 Tesla level of magnetic treatment.

At vegetative stage an increase in leaf total chlorophyll contents (Fig. 1E–F) was found due to different seed treatments except to that of at 10 kGy and 20 kGy levels of gamma radiation where non-significant decrease and 1 min treatment of He-Ne radiation where a significant decrease in leaf total chlorophyll contents was recorded. In comparison the maximum increase in leaf total chlorophyll contents was recorded in plants raised from seeds treated with different levels of He-Ne and UV radiation. At flowering stage non-significant change in total chlorophyll contents was observed in all the levels of different physical seed treatments except to that of 0.1 Tesla of magnetic treatment where a significant decrease was recorded as compared to plants raised from non-treated or hydro-primed seeds.

At vegetative stage all types of treatments increased the leaf carotenoid contents except gamma radiation where non-significant change was observed (Fig. 1G–H). In 2 and 3 min treatment level of He-Ne showed significantly higher value while in magnetic treatment only 0.1 Tesla was observed to be significantly higher. All the levels of UV treatment resulted in significantly higher values of carotenoids as compared to plants raised from non-treated or hydro primed seeds. However, at the flowering stage no effect of different treatments was found in leaf carotenoids except to that of magnetic treatments at 10 kGy where a decrease was recorded in leaf carotenoids.

Primary Metabolites

Data presented in Fig. 2 shows the influence of seed physical treatments on primary metabolites of sunflower plants grown from seeds subjected to different physical treatments,

Table 1: Response of secondary metabolites of sunflower to pre sowing seed treatment with selected physical agents

Treatment	levels	Leaf Fresh weight	Leaf Dry weight	Achene oil contents (%)	No of Achenes per cake	Cake weight (g)	100 Achenes weight (g)
Gamma	Control	1.9 ± 0.1de	0.38 ± 0.14d	14.8 ± 0def	193.67 ± 24.13f	129.33 ± 9.5ab	5.10 ± 0.08ab
	Hydroprimed	1.9 ± 0.17de	0.38 ± 0.14d	14.8 ± 0def	193.67 ± 24.13f	129.33 ± 9.5ab	5.22 ± 0.25ab
	10kGy	5.91 ± 0.41b	1.14 ± 0.19b	16.1 ± 0bc	580 ± 8.89b	90 ± 8.89bc	3.86 ± 0.07bc
	20kGy	2.94 ± 0.4de	0.57 ± 0.06cd	15.4 ± 0cdef	506.67 ± 40.41bc	157.33 ± 12.7a	2.96 ± 0.08cd
	30kGy	3.18 ± 1.16de	0.58 ± 0.07cd	17.2 ± 0a	138.67 ± 1.53f	36 ± 14.11ef	4.86 ± 0.32ab
He-Ne	Control	1.9 ± 0.1de	0.38 ± 0.14d	14.8 ± 0def	193.67 ± 24.13f	129.33 ± 9.5ab	3.10 ± 0.08ab
	Hydroprimed	1.9 ± 0.17de	0.38 ± 0.14d	14.8 ± 0cdef	193.67 ± 24.13f	129.33 ± 9.5ab	5.22 ± 0.25ab
	1 Minute	5.42 ± 0.73bc	1.04 ± 0.07bc	15.3 ± 0cdef	218 ± 17.09ef	24.33 ± 18.58e	5.78 ± 0.2a
	2 Minutes	8.16 ± 0.89a	1.69 ± 0.15bc	14.6 ± 0ghi	330 ± 17.32de	28 ± 2f	4.34 ± 0.16ab
	3 Minutes	3.62 ± 0.19cde	0.53 ± 0.05cd	15.5 ± 0cdef	207 ± 6.08ef	51 ± 3.61def	4.84 ± 0.13ab
UV	Control	1.9 ± 0.1de	0.38 ± 0.14d	14.8 ± 0def	193.67 ± 24.13f	129.33 ± 9.5ab	5.10 ± 0.08ab
	Hydroprimed	1.9 ± 0.17de	0.38 ± 0.14d	14.8 ± 0def	193.67 ± 24.13f	129.33 ± 9.5ab	5.22 ± 0.25ab
	20 Minutes	4.15 ± 0.27cd	0.62 ± 0.03cd	15.42 ± 0cdef	215 ± 0ef	63 ± 0cde	4.78 ± 0ab
	30 Minutes	3.88 ± 0.23cde	0.68 ± 0.08cd	16.1 ± 0bc	404 ± 5.29cd	67 ± 5.2cde	5.44 ± 0.25a
	40 Minutes	5.19 ± 0.16bc	0.65 ± 0.19cd	15.8 ± 0cd	330 ± 101.49de	49 ± 3.61ef	2.86 ± 0.06d
Magnetic	Control	1.9 ± 0.1de	0.38 ± 0.14d	14.8 ± 0def	193.67 ± 24.13f	129.33 ± 9.5ab	5.10 ± 0.08ab
	Hydroprimed	1.9 ± 0.17de	0.38 ± 0.14d	14.8 ± 0def	193.67 ± 24.13f	129.33 ± 9.5ab	5.23 ± 0.25ab
	0.1 Tesla	3.18 ± 0.68de	0.48 ± 0.17d	15.6 ± 0cde	583.33 ± 28.87b	85.33 ± 5.03bcd	5.58 ± 0.16a
	0.2 Tesla	4.11 ± 0.24cde	0.55 ± 0.09d	17.8 ± 0a	733.33 ± 57.74a	110.33 ±9.29abc	5.84 ± 0.14a
	0.3 Tesla	2.89 ± 0.37de	0.4 ± 0.06d	16.5 ± 0ab	350 ± 50de	104.33 ± 7.51bc	3.94 ± 0.08bc

Means (n=5 ± S.E.) followed by different letters are significantly variable at 5% level of probability

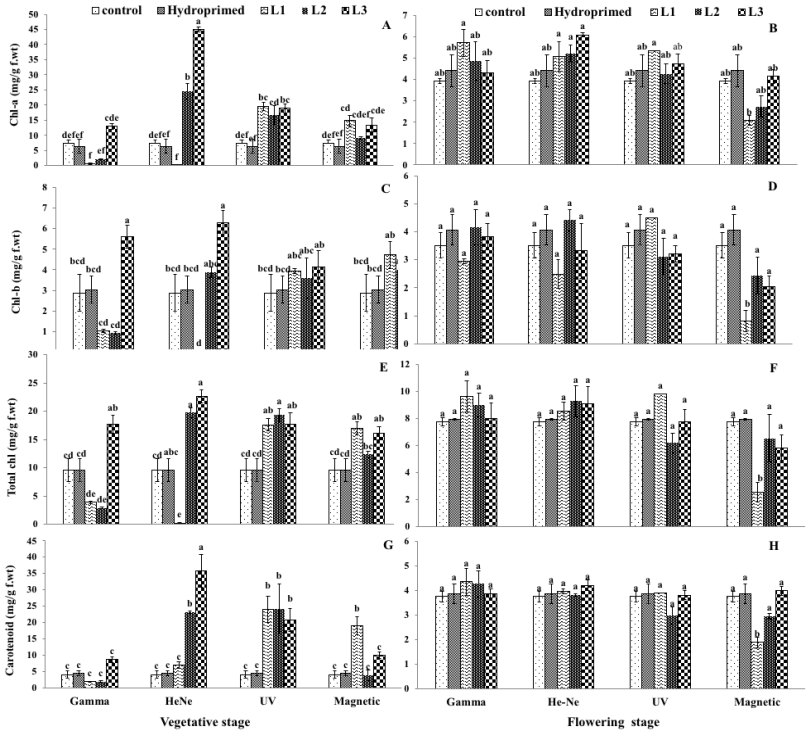

Fig. 1: Effect of pre sowing seed treatment with selected physical agents on pigmentation in sunflower. Means with same letters, for each trait, do not differ significantly from each other at $P \leq 0.05$. For magnetic field L1= 0.1 Tesla, L2= 0.2 Tesla, L3=0.3 Tesla, for ultraviolet (L1= 20 min, L2=40 min, L3= 60 min.), for He-Ne laser treatment (L1= 1 min, L2= 2 min, L3= 3 min), for gamma (γ) irradiation (L1= 10 kGy, L2=20 kGy, L3= 30 kGy). A, C, E, G corresponds to Chlorophyll a, b, total chlorophyll and carotenoid contents at vegetative stage while B, D, F, H corresponds to Chlorophyll a, b, total chlorophyll and carotenoid contents at flowering stage

estimated at the vegetative as we all as at the flowering stage. Seed treatment with all four physical treatments significantly enhanced the Total Soluble Proteins (TSP) contents at the vegetative stage (Fig. 2A) but the intensity of this increase was treatment and treatment level specific. At vegetative stage this increase in TSP in plants grown from seeds treated with gamma radiation was significantly higher at 10 kGy and 20 kGy treatment level as compared with 30 kGy relative to non-treated or hydro-primed ones. But in case of He-Ne laser all treatment levels were equally effective in increasing TSP. However, in case of UV radiation and magnetic treatment the significant increase in TSP was only at 40 min treatment and 0.1 Tesla respectively.

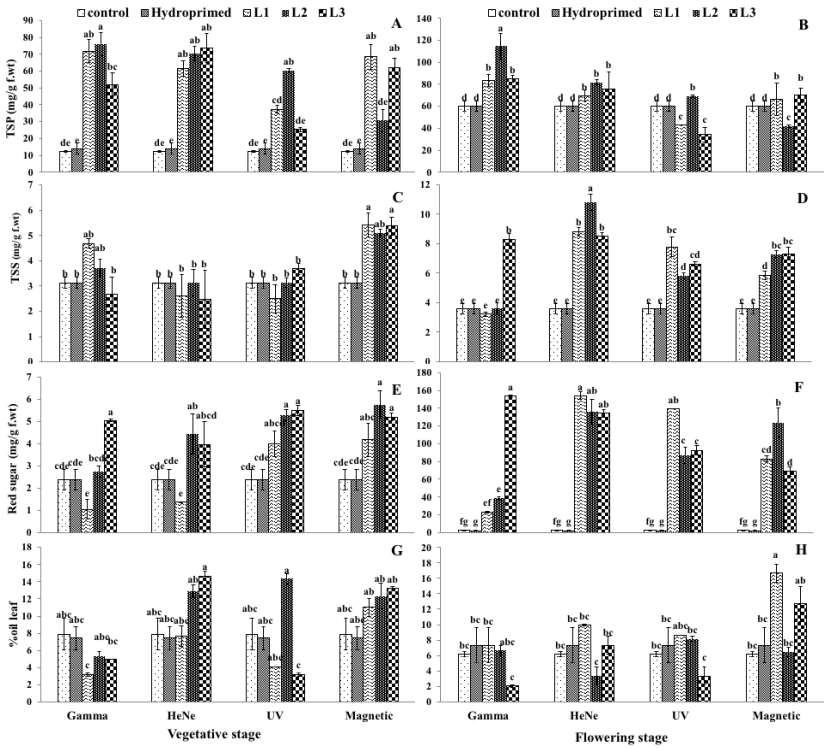

Fig. 2: Effect of pre sowing seed treatment with selected physical agents on primary metabolites in sunflower. Means with same letters, for each trait, do not differ significantly from each other at $P \leq 0.05$. For magnetic field L1= 0.1 Tesla, L2= 0.2 Tesla, L3 = 0.3 Tesla, for ultraviolet (L1= 20 min, L2=40 min, L3= 60 min), for He-Ne laser treatment (L1= 1 min, L2= 2 min, L3= 3 min), for gamma (γ) irradiation (L1= 10 kGy, L2=20 kGy, L3= 30 kGy). A, C, E, G corresponds to Total soluble proteins (TSP), Total soluble sugars (TSS), Reducing sugars and Leaf percentage oil contents at vegetative stage while B, D, F, H corresponds to Total soluble proteins (TSP), Total soluble sugars (TSS), Reducing sugars and Leaf percentage oil at flowering stage

The seed physical treatment also affected the TSP of sunflower plants at the flowering stage (Fig. 2B) but the increasing or decreasing effect was treatment and treatment level specific. All the He-Ne levels were found to be equally effective in increasing TSP but in case of gamma radiation, 20 kGy treatments were the most effective. However, in case of UV seed treatment a significant increase in TSP was recorded at 40 min treatment and a significant decrease was found at 20 and 60 min treatment levels. Similarly, in case of magnetic treatment a significant increase in TSP was recorded at 0.1 Tesla and 0.3 Tesla levels but the opposite was true for 0.2 Tesla treatment level.

The Total Soluble Sugars (TSS) of sunflower plants at vegetative stage showed an increasing effect in plants raised from seeds treated with magnetic treatment (Fig. 2C). In this treatment all three levels significantly increased the TSS and found equally effective except 0.2 Tesla which showed non-significant increase. At flowering stage, the improvement in TSS was found due to all physical treatments (Fig. 2D). In case of gamma radiation this improvement in TSS was recorded only at 30 kGy level but in case of He-Ne, UV and magnetic treatment, all three treatment levels improved the leaf TSS of sunflower plants.

At vegetative stage significant increase in Leaf Reducing sugars (LRS) was recorded in plants raised from seeds treated with 30 kGy level of gamma and 2 min level of He-Ne radiation (Fig. 2E). However, in case of UV and magnetic treatment all higher levels were found effective in increasing the LRS and increased with increasing the treatment level. Moreover, at the flowering stage the significant increase in LRS was found due to all physical treatments and all the levels were found equally effective (Fig. 2F).

At vegetative stage all the treatments showed non-significant differences in Leaf Oil contents from their non treated or hydro primed counterparts. A non-significant decrease in Percentage oil was found due to treatments of gamma and UV radiations at all levels except to that of 40 min level of UV where slight increase was recorded in oil percentage contents (Fig. 2G). However, He-Ne and magnetic seed treatment non significantly improved the oil contents at all the levels except 1 min treatment of He-Ne where non-significant change was noted. Similarly, at the flowering stage, the increase or decrease in oil percentage was treatment and treatment level specific. A significant increase was recorded only in 0.1 Tesla magnetic treatment at flowering stage while all other treatment and treatment levels showed non-significant increase except 30 kGy gamma and 60 min of UV as well as 2 min He-Ne treatment showed slight decrease (Fig. 2H).

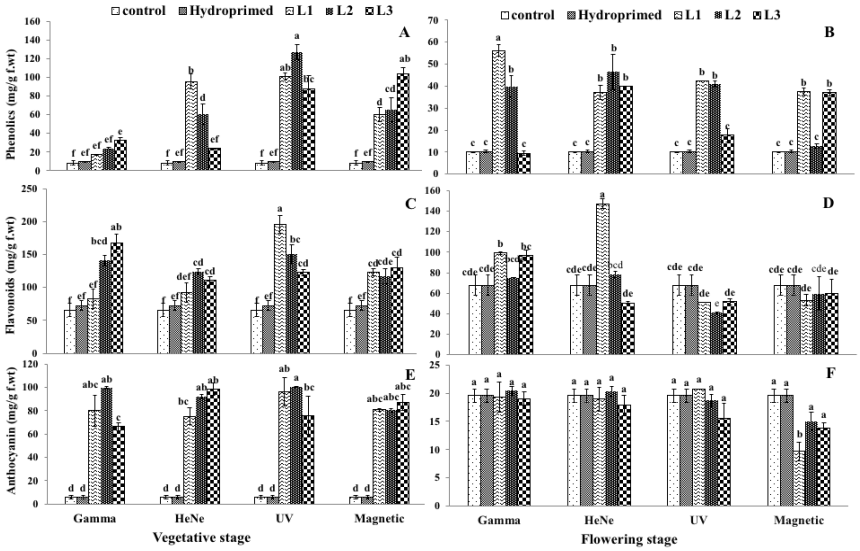

Fig. 3: Effect of pre sowing seed treatment with selected physical agents on secondary metabolites in sunflower. Means with same letters, for each trait, do not differ significantly from each other at $P \leq 0.05$. For magnetic field L1= 0.1 Tesla, L2= 0.2 Tesla, L3=0.3 Tesla, for ultraviolet (L1= 20 min, L2=40 min, L3= 60 min), for He-Ne laser treatment (L1= 1 min, L2= 2 min, L3= 3 min), for gamma (γ) irradiation (L1= 10 kGy, L2=20 kGy, L3= 30 kGy). A, C, E corresponds to Phenolics, Flavonoids and Anthocyanin at vegetative stage while B, D, F corresponds to Phenolics, Flavonoids and Anthocyanin at flowering stage

Secondary Metabolites

Secondary Metabolites contents increased significantly due to different physical seed treatment at all applied levels (Fig. 3). Leaf phenolic contents underwent an improvement in leaf phenolics in plants raised from seed treated with 10 kGy gamma and 1 min treatment of He-Ne radiation at the vegetative stage (Fig. 3A). At flowering stage all seed treatments improved the leaf phenolics contents except to that of 10 kGy gamma and 0.2 Tesla magnetic treatments respectively, where no increasing or decreasing effect was recorded on leaf phenolics and in UV treatment for 60 min where non-significant increase in this attribute was observed (Fig. 3B).

Leaf flavonoid contents showed increasing trend was observed in all the treatments at vegetative stage (Fig. 3C–D). The maximum increase was recorded in plants raised from seeds treated with different levels of UV radiation followed by gamma radiation. However, this increase was observed to be level specific. All the treatment levels showed significant increase except to that of 10 kGy of both gamma and He-Ne treatment for 1 min which showed non-significant increase in flavonoid contents. At flowering stage this increase was found at 10 kGy levels of gamma and 1 min He-Ne radiation.

In case of leaf anthocyanin contents (Fig. 3E–F) all types of treatments were found equally effective. However, at the flowering stage no effect on leaf anthocyanin contents was found due to different physical treatment except to that of magnetic seed treatment where a decrease in leaf anthocyanin contents was recorded at 0.1Tesla.

Discussion

Gamma radiation, a type of electromagnetic waves has potential to penetrate into cells and can bring about massive change in plant structure and metabolism (U.N.S.C., 2000). In the present study plants raised from seeds treated with gamma radiation increased the primary metabolites such as chlorophyll a and total chlorophyll contents, total soluble proteins and sugars. The observed increase may be attributed directly to either by interfering the cellular metabolism regulation or genome modifications (especially on the genes controlling these traits) (Dhakshanamoorthy *et al.,* 2011; Macovei *et al.,* 2014; Le *et al.,* 2019). Previously it has been extensively reported that gamma radiation treatment may modulate hormonal signaling or stimulate enzymatic efficiency which is usually linked with increase in total soluble proteins (Dhakshanamoorthy *et al.,* 2011). The findings of current study support this phenomenon. The distinct rise of chlorophyll contents in plants exposed to gamma radiation possibly might be a consequence of recuperation of plant after exposure (Borzouei *et al.,* 2010; Marcu *et al.,* 2013a). An increase in flavonoids and anthocyanin etc. was also observed in response to gamma radiation treatment. The availability of metabolites results in growth and yield attributes especially number of achenes per cake and seed oil contents. Increase in number of achenes per cake and percentage oil contents in response to lower doses of gamma radiation in this field study may be attributed to increase in pigmentation observed in this study, which in turn has been observed to increase primary metabolites, ultimately resulting in better growth and yield.

Thus, the response is might be at physiological level instead of genetic. Moreover, the role of plant growth hormones could also be critical (Singh and Datta, 2009). The results are in accordance with previous finding of Wi *et al*. (2007) who observed increase in pigmentation and antioxidant potentials which may ultimately lead to enhanced growth and yield.

The gamma radiation effects have been observed to dose specific. While treating plants with gamma irradiations it is generally prescribed by researchers that only the low doses of application promptly increased the many growth yield and biochemical attribute of agronomical crops (Dhakshanamoorthy *et al*., 2011; Marcu *et al.*, 2013b) while the radiation dose above 100 Gy resulted in an obvious decline in chlorophyll a, b and total chlorophyll contents (Borzouei *et al.*, 2010). Our study also revealed the same as sunflower plants raised from seeds treated with at 30 kGy dose of gamma radiation, although at vegetative stage, while the still lower doses showed increased pigmentation at flowering stage. The gamma treatment also improved some yield attributes like number of achenes and seed oil percentage. Our research also supports the fact that 10 kGy and 20 kGy of gamma radiation was effective in primary metabolite modulation. 30 kGy however caused significant change only in secondary metabolites which may be attributed to triggering of plant defense mechanism.

In recent years, the development of laser treatments has been highlighted significantly due to its documentation as stress tolerance agent in plants. Researchers have recorded positive assimilation of growth in plants and its metabolism due to He-Ne laser treatment and its role under stress (Qiu *et al.*, 2010). The similar increase in growth and pigmentation in wheat were observed by Chen *et al.* (2005). As per experimentation with cucumber, seeds irradiated with He-Ne laser generated plants showed significant increase in root growth, photosynthesis and even in yield Asghar *et al.* (2016). Our study also showed similar enhancement of photosynthetic pigments at vegetative stage (1 and 2 min) but not at flowering stage, possibly due to maturity of plant. Improved leaf biomass, and phenolic content displayed by this application additionally (Qiu *et al.*, 2008). Yield, the ultimate objective also improved as number of achenes per cake and oil contents underwent significant increase. Although the up-regulated physiological processes of growth, photosynthesis and antioxidation might be attributed to modified expressions of the related genes induced by seed pretreatment with He-Ne laser (Qiu *et al.*, 2017) but still the mechanism of how He-Ne laser improved the growth and biochemical attributes need to be further addressed.

Effect of UV treatment has also been found (Ouhibi *et al*., 2014) to be dose dependent with minimum doses displaying better seedling establishment. Likewise, in our study, medium ranged dose amplified leaf biomass accumulation in sunflower study. Although UV-B radiation is well documented due to its injurious effects on plant different morpho-physiological aspects, most of them are related to DNA malfunctioning, damage of protein and membrane structures that ultimately slow or stop the process of photosynthesis (Hideg *et al.*, 2013) but in our study UV irradiation for 40 min triggered the rate of protein synthesis in vegetative as well as in flowering stage of sunflower. Our study also showed increase in yield aspects such as number of achenes and oil percentage. These results are supported by Shaukat *et al*. (2013) who observed increased phenolics, and other antioxidants in response to ultraviolet treatment, making it suitable for further study, similar findings were made for UV in current experiment. Consequently, responses to such application would be either stimulatory or inhibitory that would ultimate effect upon plant over all biomass production in positive or negative way (Verdaguer *et al.*, 2017).

Magnetic field activity stimulates the activity of proteins and enzymes, and may enhance the efficiency of metabolic precursors including photosynthetic pigments (Iqbal *et al.*, 2012) due to orientation of their unpaired electrons (Kordas, 2002). Enhanced generation of metabolites may result in activation of other metabolic pathways ending with fruitful outcomes. In our study, in response to magnetic treatment, % oil was recorded in a better mood. Further, chlorophyll contents were higher at vegetative stage presenting the application stage specific too. Moreover, magnetic application in the range of 0.1 and 0.2 T improved leaf fat, seed oil, total phenolics, showing not only the synchronous with previous findings (Poinapen *et al.*, 2013).

Further investigations of the mechanism responsible for pre-sowing physical treatment induced metabolite modulations, may provide an efficient approach for developing strategies to raise plants with not only strong plant defense but also the yield with maximum accumulation of nutrients in edible plant parts. The observed differential modification response showed existence of different mechanisms in sunflower to alter the metabolic profile and should be subjected to future studies.

Conclusion

Sunflower responded to all the pre-sowing physical treatments which were applied, however, plants grown from seeds treated with 10 kGy and 20 kGy gamma irradiation observed more improved growth and yield compared to plants raised from untreated seeds or treated with other physical treatments. The most effective dose of gamma radiation was observed to be 10 kGy. The enhanced growth and yield were observed to be due to modulation of pigmentation, primary metabolites and secondary metabolites.

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