INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 15, No. 6, p. 174-185, 2019

RESEARCH PAPER OPEN ACCESS

Exploring the mutagenic properties of picric acid on some biochemical attributes of three *Brassica napus* L. cultivars

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Key words: Canola cultivars, Picric acid treatments, Soaking durations, Significance.

http://dx.doi.org/10.12692/ijb/15.6.174-185 Article published on December 18, 2019

Abstract

The present study was carried out to explore mutagenic effect picric acid on some biochemical parameters including carotenoid, phenols, prolines, protein and sugar contents of three canola cultivars (*Abasin-95*, *Dur e Nifa*, *Nifa Gold*). Three factorial randomized complete block design was selected for the present study. Significant variation in the results of carotenoid, phenols, prolines, and protein and sugar contents in the leaves of all verities was observed using different treatment levels of picric acid. Similarly, priming durations also exhibited significant results for all the parameters except leaf phenols and proteins contents. Moreover, ANOVA for inter cultivar variations was also found significant for various biochemical parameters of the three canola cultivars excluding protein contents. These results suggest that all the three cultivars possess different genotypes. It is obvious from the data that picric acid treatment levels reduced the leaf carotenoid and phenol contents of canola plants as compared to control. On the other hand, lower doses of picric acid were stimulatory for leaf proline and sugar contents. Among different picric acid priming durations, plants raised from seeds pre-soaked with 6 hours duration proved good for all the studied parameters except leaf sugar contents. Cultivar *Abasin*-95 superseded rest of the cultivars in leaf proline and sugar contents while cultivar *Dur e Nifa* and cultivar *Nifa Gold* showed maximum leaf carotenoid and phenol contents respectively. The high production of such stress combating metabolites indicate that these canola cultivars possess strong genes which can cope with adverse environment and is suggested for cultivation as an alternate crop in plant challenging conditions.

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Introduction

The genus *Brassica* is the most important and prominent one with in the family Brassicaceae, which includes some very well-known economically important crops. *Brassica* species and varieties are commonly used for food (broccoli, cauliflower, cabbage, Choy sum, rutabaga, turnip and production of oil (canola and mustard). Brassica foods are very nutritive, providing nutrients and health-promoting phytochemicals such as vitamins, carotenoids, fiber, soluble sugars, minerals, glucosinolates and phenolic compounds (Podsedek,

2007; Jahangir *et al.*, 2009).

Phenolics and carotenoids are a large group of phytochemicals wide spread in the plant kingdom. Phenolics and carotenoids have received considerable attention for being potentially protective factors against cancer and heart diseases, in part because of their potent antioxidative properties and their ubiquity in a wide range of commonly consumed foods of plant origin (Altemimi *et al.*, 2017). So consuming plants rich in phenolics and carotenoids could overcome and cancel out the hazards of various food carcinogens which human are taking in large amount on daily basis (Aremu and Nweze, 2017). When exposed to stressful conditions, plants accumulate an array of metabolites, particularly amino acids and soluble sugars. Amino acids have traditionally been considered as precursors to and constituents of proteins and play an important role in plant metabolism and development. Proline, an amino acid, plays a highly beneficial role in plants exposed to various stress conditions. Besides acting as an excellent osmolyte, proline plays three major roles during stress, i.e., as a metal chelator, an antioxidative defense molecule and a signaling molecule (Naidu *et al.*, 1991; Bassi and Sharma 1993; Hare and Cress 1997; Hare and Cress 1998; Rhodes *et al.*, 2002; Munns 2005; Sharma and Dietz 2006). Review of the literature indicates that a stressful environment results in an over production of proline in plants which in turn imparts stress tolerance by maintaining cell turgor or osmotic balance; stabilizing membranes thereby preventing electrolyte leakage; and bringing concentrations of reactive oxygen species (ROS) with in normal ranges, thus preventing oxidative burst in plants (Jahangir *et al.*, 2012).

In the present study, effort has been made to turn on the nutrients producing genes to produce carotenoids and phenolics along with proline, protein and sugars in enough in canola plant using a least explored chemical mutagen, Picric acid, a well-known compound used in explosives (Fig. 1).

Materials and methods

The experiment was based on 3 factors factorial randomized complete block design (first factor: picric acid concentrations, second factor: Priming durations, Third factor: Three canola cultivars), with three replications. Seeds of three canola cultivars (*Abasin-95, Dur e Nifa* and *Nifa Gold*) were obtained from Nuclear Institute for Agriculture, Peshawar (NIFA). Prior to start the experiment seeds viability were tested. After positive seed viability test, seeds of the three canola cultivars were soaked in 5 concentrations (0, 5 mM, 10mM, 15mM, 20mM) of picric acid solution for three different time durations (3 hours, 6 hours, 9 hours) and sown in pots filled of soil. The soil was prepared by mixing clay and loam in nearly equal amounts.

After germination, seedlings were allowed to grow to become mature plants. At mature age leaves were collected from plants in each pot. And leaf carotenoid contents were determined for each pot following protocol of Arvayo-Enríquez *et al.* (2013). Similarly leaf phenol contents were determined following the method of Mahadevan and Sridhar (1986). For the determination of leaf proline contents protocol of Bates *et al.*, 1973 were followed.

The methods of Lowery *et al.* (1951) and Dubois *et al.* (1956) were followed for the determination of leaf protein and leaf sugar contents. Fisher analysis of variance technique (1985) and LSD test at 5% probability was applied on the data to compare the differences among treatments, priming and cultivars means (Steel and Torie, 1984).

Results and discussion

Effect on leaf carotenoid contents (mg/gm)

ANOVA for picric acid treatment levels (A), priming durations (B), three canola cultivars (C) and their all interactions (A×B, B×C, A×C, A×B×C) exhibited significant differences for leaf carotenoid contents (Table 1).

Table 1. Table for mean squares of leaf carotenoid contents (mg/gm), leaf phenol contents (mg/ml), leaf proline contents (mg/gm), leaf protein contents (mg/gm) and leaf sugar contents (mg/gm).

Source	Degree of freedom	Carotenoid contents	Phenol contents	Proline contents	Protein contents	Sugar contents
Replication	2	0.000	0.003	0.000	0.000	0.000
Treatment levels (A)	4	0.278^{S}	0.026^{S}	0.001^{S}	0.004^{NS}	0.001^{S}
Priming durations (B)	2	0.011^{S}	0.003^{NS}	0.074^{S}	0.004^{NS}	0.030^{S}
A×B	8	0.173^{S}	0.023^{S}	0.004^{NS}	0.006^{NS}	0.001^{S}
Canola cultivars (C)	2	7.413^{S}	0.705^{S}	0.001^{S}	0.000^{NS}	0.000^{S}
A×C	8	0.060^{S}	0.017^{S}	0.001^{NS}	0.000^{NS}	0.001^{S}
B×C	4	0.575^{S}	0.047^{S}	0.001^{S}	0.000^{NS}	0.000^{S}
A×B×C	16	0.049^{S}	0.019^{S}	0.000^{NS}	0.001^{NS}	0.000^{S}
Error	88	0.001	0.004	0.001	0.001	0.001

S for Significant NS for Non-significant.

Table 2. Effect of various concentrations (0, 5 mM, 10 mM, 15 mM, 20 mM) and different priming durations (3 hours, 6 hours, 9hours) of picric acid on leaf carotenoid contents (mg/gm) of three canola cultivars.

Treatment Levels (A) × Priming Levels (B) × Canola cultivars Interaction (C)				
A×B×C		*Abasin-95*	*Dur e Nifa*	*Nifa Gold*
Control	3 hours	1.039^{ab}	1.012^{abc}	1.057^{a}
5 Mm		0.361^{l}	1.001^{bcd}	0.098^{n}
10 mM		0.339^{l}	0.969^{cd}	0.085^{no}
15 mM		0.343^{l}	0.991^{bcd}	0.076^{nop}
20 mM		0.356^{l}	0.898^{fg}	0.090^{n}
Control	6 hours	0.851^{ghi}	0.899^{fg}	0.239^{m}
5 mM		0.865^{fgh}	0.875^{fg}	0.014^{q}
10 mM		0.790^{j}	0.860^{fghi}	0.010^{q}
15 mM		0.685^{k}	0.961^{cd}	0.069^{nop}
20 mM		0.958^{de}	0.909^{ef}	0.198^{m}
Control	9 hours	0.980^{cd}	0.896^{fg}	0.029^{pq}
5 mM		0.865^{fgh}	0.816^{hij}	0.013^{q}
10 mM		0.990^{bcd}	0.859^{fghi}	0.01^{q}
15 mM		0.968^{cd}	0.810^{ij}	0.034^{opq}
20 mM		0.888^{fg}	0.795^{j}	0.032^{pq}
Treatment Levels (A) × Priming Levels (B) Interaction				
A×B	3 hours	6 hours	9 hours	Treatment Levels Means
Control	1.036^{a}	0.663^{bc}	0.635^{cd}	0.778^{a}
5 mM	0.487^{i}	0.585^{fg}	0.565^{gh}	0.545^{c}
10 mM	0.464^{ij}	0.553^{h}	0.620^{de}	0.546^{c}
15 mM	0.470^{ij}	0.572^{gh}	0.604^{ef}	0.549^{c}
20 mM	0.448^{j}	0.688^{b}	0.572^{gh}	0.599^{b}
Priming Levels (B) × Canola cultivars (C) Interaction				
B×C	*Abasin-95*	*Dur e Nifa*	*Nifa Gold*	Priming Levels Means
3 hours	0.488^{e}	0.974^{a}	0.281^{f}	0.581^{b}
6 hours	0.830^{d}	0.901^{c}	0.106^{g}	0.612^{a}
9 hours	0.938^{b}	0.835^{d}	0.024^{h}	0.599^{a}
Treatments Levels (A) × Canola cultivars (C) Interaction				
A×C	Control	5 mM	10 mM	15 mM	20 mM	Cultivars Means
Abasin-95	0.957^{a}	0.607^{f}	0.706^{ef}	0.665^{g}	0.734^{e}	0.752^{b}
Dur e Nifa	0.936^{ab}	0.897^{c}	0.896^{cd}	0.921^{bc}	0.867^{d}	0.903^{a}
Nifa Gold	0.442^{h}	0.042^{j}	0.035^{j}	0.060^{j}	0.107^{i}	0.137^{c}

Lsd value at 5 % probability level of significance for Picric acid treatment levels = 0.01710, priming levels = 0.01325, cultivars = 0.01325, Picric acid treatment levels × priming levels = 0.02962, priming levels × cultivars = 0.02295, Picric acid treatment levels ×cultivar = 0.02962 and Picric acid treatment levels × priming levels × cultivars =0.05131. Values bearing similar letters in rows and column are statistically non-significant.

Among picric acid treatment levels means, maximum mean value for leaf carotenoid contents was recorded for control. All the picric acid treatment levels (5 mM, 10 mM, 15 mM, 20 mM) means were found to be less than that of the control. Among picric acid treatment levels, 5 mM, 10 mM, and 15 mM concentrations showed non-significantly different mean values of 0.545, 0.546 and 0.549 respectively. Picric acid treatment level of 20 mM showed maximum mean value of 0.599 for leaf carotenoid contents which was higher and significantly different from other treatments levels except control.

Table 3. Effect of various concentrations (0, 5 mM, 10 mM, 15 mM, 20 mM) and different priming durations (3 hours, 6 hours, 9 hours) of picric acid on leaf phenol contents (mg/ml) of three canola cultivars.

Treatment Levels (A) × Priming Levels (B) × Canola cultivars Interaction (C)				
A×B×C		*Abasin-95*	*Dur e Nifa*	*Nifa Gold*
Control	3 hours	0.029^{ij}	0.038^{ij}	0.400^{b}
5 mM		0.013^{j}	0.039^{ij}	0.301^{bcdef}
10 mM		0.011^{j}	0.303^{bcdfg}	0.385^{b}
15 mM		0.034^{ij}	0.350^{bcd}	0.360^{bc}
20 mM		0.021^{ij}	0.339^{bcde}	0.389^{b}
Control	6 hours	0.196^{gh}	0.251^{defg}	0.275^{cdefg}
5 mM		0.080^{ij}	0.267^{cdefg}	0.251^{defg}
10 mM		0.099^{hij}	0.251^{defg}	0.281^{cdefg}
15 mM		0.101^{hij}	0.233^{fg}	0.235^{fg}
20 mM		0.121^{hi}	0.260^{cdefg}	0.245^{efg}
Control	9 hours	0.011^{j}	0.321^{bcdf}	0.523^{a}
5 mM		0.021^{ij}	0.106^{hij}	0.281^{cdefg}
10 mM		0.103^{hij}	0.010^{j}	0.273^{cdefg}
15 mM		0.099^{hij}	0.250^{defg}	0.269^{cdefg}
20 mM		0.080^{ij}	0.259^{cdefg}	0.278^{cdefg}

Treatment Levels (A) × Priming Levels (B) Interaction				
A×B	3 hours	6 hours	9 hours	Treatment Levels Means
Control	0.156^{def}	0.241^{abc}	0.285^{a}	0.227^{a}
5 mM	0.118^{f}	0.199^{bcd}	0.136^{ef}	0.151^{c}
10 mM	0.233^{abc}	0.210^{bcd}	0.129^{f}	0.191^{b}
15 mM	0.248^{abc}	0.190^{cde}	0.206^{bcd}	0.215^{ab}
20 mM	0.250^{ab}	0.209^{bcd}	0.206^{bcd}	0.221^{ab}

Priming Levels (B) × Canola cultivars (C) Interaction				
B×C	*Abasin-95*	*Dur e Nifa*	*Nifa Gold*	Priming Levels Means
3 hours	0.022^{e}	0.214^{bc}	0.367^{a}	0.201
6 hours	0.119^{d}	0.252^{b}	0.257^{b}	0.210
9 hours	0.063^{e}	0.189^{c}	0.325^{a}	0.192

Treatments Levels (A) × Canola cultivars (C) Interaction						
A×C	Control	5 mM	10 mM	15 mM	20 mM	Cultivars Means
Abasin-95	0.079^{ef}	0.038^{f}	0.071^{f}	0.078^{f}	0.074^{f}	0.068^{c}
Dur e Nifa	0.203^{c}	0.137^{de}	0.188^{cd}	0.278^{b}	0.286^{b}	0.218^{b}
Nifa Gold	0.399^{a}	0.278^{b}	0.313^{b}	0.288^{b}	0.304^{b}	0.316^{a}

Lsd value at 5 % probability level of significance for Picric acid treatment levels = 0.03421, cultivars = 0.02650, Picric acid treatment levels ×priming levels = 0.05925, priming levels × cultivars = 0.04589, Picric acid treatment levels ×cultivar = 0.05925 and picric acid treatment levels × priming levels × cultivars = 0.1026. Values bearing similar letters in rows and column are statistically non-significant.

Among different priming durations (3 hours, 6 hours, 9 hours) means, maximum mean value for leaf carotenoid contents was recorded in plant leaves raised from 6 hours (0.617) and 9 hours (0.599) primed seeds. The differences between mean values recorded from plant leaves raised from seeds primed for 6 hours and 9 hours were non-significantly different from each other. 0.581 was the lowest mean value recorded for leaf carotenoid contents from plants raised from seeds primed for 3 hours which showed significant differences from plants leaves raised from 6 hours and 9 hours primed seeds.

Table 4. Effect of various concentrations (0, 5 mM, 10 mM, 15 mM, 20 mM) and different priming durations (3 hours, 6 hours, 9hours) of picric acid on leaf proline contents (mg/gm) of three canola cultivars.

Treatment Levels (A) × Priming Levels (B) × Canola cultivars Interaction (C)				
A×B×C		*Abasin-95*	*Dur e Nifa*	*Nifa Gold*
Control	3 hours	0.368^{abc}	0.368^{abc}	0.365^{abcd}
5 mM		0.350^{abcdef}	0.358^{abcde}	0.362^{abcde}
10 mM		0.345^{jk}	0.349^{abcde}	0.389^{bcdefg}
15 mM		0.362^{ghijk}	0.371^{ab}	0.321^{abcde}
20 mM		0.350^{abcdef}	$0.320^{cdefghij}$	0.359^{abcde}
Control	6 hours	0.391^{ab}	0.382^{ab}	0.350^{abcdef}
5 mM		0.362^{abcde}	0.386^{abcde}	0.275^{ab}
10 mM		0.399^{abcdef}	0.341^{ab}	0.299^{a}
15 mM		0.311^{abc}	$0.315^{cdefghij}$	$0.291^{efghijk}$
20 mM		0.368^{abc}	0.355^{abcde}	$0.329^{cdefghi}$
Control	9 hours	0.267^{k}	0.299^{fghijk}	0.280^{ijk}
5 mM		0.270^{ab}	0.276^{jk}	0.359^{jk}
10 mM		$0.296^{bcdefgh}$	0.296^{fghijk}	0.381^{ghijk}
15 mM		$0.291^{defghijk}$	0.296^{hijk}	0.293^{hijk}
20 mM		0.299^{fghijk}	0.299^{fghijk}	0.285^{ijk}

Treatment Levels (A) × Priming Levels (B) Interaction				
A×B	3 hours	6 hours	9 hours	Treatment Levels Means
Control	0.367^{ab}	0.374^{a}	0.282^{e}	0.341^{a}
5 mM	0.357^{ab}	0.378^{a}	0.274^{e}	0.336^{ab}
10 mM	0.351^{ab}	0.376^{a}	0.297^{de}	0.341^{a}
15 mM	0.371^{ab}	0.316^{cd}	0.293^{de}	0.327^{bc}
20 mM	0.343^{bc}	0.351^{ab}	0.294^{de}	0.329^{ab}

Priming Levels (B) × Canola cultivars (C) Interaction				
B×C	*Abasin-95*	*Dur e Nifa*	*Nifa Gold*	Priming Levels Means
3 hours	0.362^{a}	0.351^{a}	0.361^{a}	0.358^{a}
6 hours	0.366^{a}	0.367^{a}	0.344^{a}	0.359^{a}
9 hours	0.286^{b}	0.292^{b}	0.286^{b}	0.288^{b}

Treatments Levels (A) × Canola cultivars (C) Interaction						
A×C	Control	5 mM	10 mM	15 mM	20 mM	Cultivars Means
Abasin-95	0.342	0.329	0.349	0.331	0.339	0.338^{a}
Dur e Nifa	0.350	0.338	0.347	0.323	0.325	0.336^{a}
Nifa Gold	0.332	0.341	0.329	0.326	0.324	0.330^{a}

Lsd value at 5 % probability level of significance for Picric acid treatment levels = 0.01710, priming levels = 0.1325, cultivars = 0.01325, Picric acid treatment levels × priming levels = 0.02962, priming levels × cultivars = 0.02295 and Picric acid treatment levels × priming levels × cultivars = 0.05131. Values bearing similar letters in rows and column are statistically non-significant.

In case of three canola cultivars (*Abasin-95*, *Dur e Nifa* and *Nifa Gold*), *Dur e Nifa* was found to show maximum mean value of 0.903 for leaf carotenoid contents which was significantly different from cultivar *Abasin-95* (0.752) and cultivar *Nifa Gold* (0.137). Mean value for leaf carotenoid contents recorded from cultivar *Abasin-95* was significantly higher than cultivar *Nifa Gold*.

Table 5. Effect of various concentrations (0, 5 mM, 10 mM, 15 mM, 20 mM) and different priming durations (3 hours, 6 hours, 9hours) of picric acid on leaf protein contents (mg/gm) of three canola cultivars.

Treatment Levels (A) × Priming Levels (B) × Canola cultivars Interaction (C)				
A×B×C		*Abasin-95*	*Dur e Nifa*	*Nifa Gold*
Control	3 hours	0.589	0.560	0.538
5 mM		0.549	0.565	0.560
10 mM		0.589	0.591	0.599
15 mM		0.541	0.551	0.565
20 mM		0.589	0.580	0.579
Control	6 hours	0.599	0.590	0.590
5 mM		0.538	0.531	0.536
10 mM		0.561	0.601	0.615
15 mM		0.620	0.600	0.601
20 mM		0.599	0.589	0.560
Control	9 hours	0.601	0.629	0.631
5 mM		0.624	0.599	0.591
10 mM		0.609	0.603	0.580
15 mM		0.571	0.581	0.534
20 mM		0.539	0.549	0.588

Treatment Levels (A) × Priming Levels (B) Interaction				
A×B	3 hours	6 hours	9 hours	Treatment Levels Means
Control	0.562	0.593	0.620	0.592
5 mM	0.558	0.535	0.605	0.566
10 mM	0.593	0.592	0.597	0.594
15 mM	0.552	0.607	0.562	0.574
20 mM	0.583	0.583	0.559	0.575

Priming Levels (B) × Canola cultivars (C) Interaction				
B×C	*Abasin-95*	*Dur e Nifa*	*Nifa Gold*	Priming Levels Means
3 hours	0.571	0.569	0.568	0.570
6 hours	0.583	0.582	0.580	0.582
9 hours	0.589	0.592	0.585	0.589

Treatments Levels (A) × Canola cultivars (C) Interaction						
A×C	Control	5 mM	10 mM	15 mM	20 mM	Cultivars Means
Abasin-95	0.596	0.570	0.586	0.577	0.576	0.581
Dur e Nifa	0.593	0.565	0.598	0.577	0.573	0.581
Nifa Gold	0.586	0.562	0.598	0.567	0.576	0.578

In case of interaction of treatment levels × priming durations (A×B), maximum mean value (1.036) for leaf carotenoid contents was recorded in the control of 3 hours priming duration. On the other hand, in priming durations × canola cultivars interaction (B×C), maximum mean value (0.974) for leaf carotenoid contents was shown by cultivar *Dur e Nifa* in 3 hours priming duration.

While Minimum mean value for leaf carotenoid contents was found to be 0.024 which was recorded for cultivar *Nifa Gold* in 9 hours priming duration.

Table 6. Effect of various concentrations (0, 5 mM, 10 mM, 15 mM, 20 mM) and different priming durations (3 hours, 6 hours, 9hours) of picric acid on leaf sugar contents (mg/gm) of three canola cultivars.

Treatment Levels (A) × Priming Levels (B) × Canola cultivars Interaction (C)				
A×B×C		*Abasin-95*	*Dur e Nifa*	*Nifa Gold*
Control	3 hours	0.969^{abcdefghi}	0.965^{abcdefghi}	0.967^{abcdefghi}
5 mM		0.971^{abcdefgh}	0.980^{abcdefg}	0.991^{abc}
10 mM		1.000^{a}	0.995^{ab}	0.976^{abcdefgh}
15 mM		0.951^{abcdefghij}	0.956^{abcdefghi}	0.953^{abcdefghi}
20 mM		0.989^{abcd}	0.981^{abcdef}	0.980^{abcdefg}
Control	6 hours	0.949^{abcdefghij}	0.942^{cdefghij}	0.945^{bcdefghij}
5 mM		0.939^{defghij}	0.941^{cdefghij}	0.938^{defghij}
10 mM		0.929^{ghij}	0.937^{efghij}	0.929^{ghij}
15 mM		0.927^{hij}	0.930^{fghij}	0.941^{cdefghij}
20 mM		0.945^{bcdefghij}	0.900^{j}	0.919^{ij}
Control	9 hours	0.992^{abc}	0.959^{abcdefghi}	0.982^{abcde}
5 mM		0.999^{a}	0.998^{a}	0.998^{a}
10 mM		0.981^{abcdef}	0.988^{abcde}	0.986^{abcde}
15 mM		0.970abcdefghi	0.978abcdefgh	0.981^{abcdef}
20 mM		0.988^{abcde}	0.970^{abcdefghi}	0.965^{abcdefghi}

Treatment Levels (A) × Priming Levels (B) Interaction				
A×B	3 hours	6 hours	9 hours	Treatment Levels Means
Control	0.967^{bcde}	0.945^{defg}	0.978^{abc}	0.963^{ab}
5 mM	0.981^{abc}	0.939^{efg}	0.998^{a}	0.973^{a}
10 mM	0.990^{ab}	0.932^{fg}	0.985^{ab}	0.969^{ab}
15 mM	0.953^{cdef}	0.933^{fg}	0.976^{abc}	0.954^{b}
20 mM	0.983^{ab}	0.921^{g}	0.974^{abcd}	0.960^{ab}

Priming Levels (B) × Canola cultivars (C) Interaction				
B×C	*Abasin-95*	*Dur e Nifa*	*Nifa Gold*	Priming Levels Means
3 hours	0.976^{a}	0.975^{a}	0.973^{a}	0.975^{a}
6 hours	0.938^{b}	0.930^{b}	0.934^{b}	0.934^{b}
9 hours	0.986^{a}	0.979^{a}	0.982^{a}	0.982^{a}

Treatments Levels (A) × Canola cultivars (C) Interaction						
A×C	Control	5 mM	10 mM	15 mM	20 mM	Cultivars Means
Abasin-95	0.970^{a}	0.970^{a}	0.970^{a}	0.949^{a}	0.974^{a}	0.967a
Dur e Nifa	0.955^{a}	0.973^{a}	0.973^{a}	0.955^{a}	0.950^{a}	0.961a
Nifa Gold	0.965^{a}	0.976^{a}	0.964^{a}	0.958^{a}	0.955^{a}	0.963a

Lsd value at 5 % probability level of significance for Picric acid treatment levels = 0.01710, priming levels = 0.1325, cultivars = 0.01325, Picric acid treatment levels ×priming levels = 0.02962, priming levels × cultivars = 0.02962 and Picric acid treatment levels × priming levels × cultivars = 0.05131. Values bearing similar letters in rows and column are statistically non-significant.

In other interaction of treatment levels × canola cultivars (A×C), control of cultivar *Abasin-95* showed maximum mean value (0.957) for leaf carotenoid contents. While for the same, minimum mean values (0.035) was shown by 10 mM 10 picric acid treatment level of cultivar *Nifa Gold*. In the combine interaction of all three factors, A×B×C (treatment levels × priming durations × canola cultivars), maximum mean value (1.057) for leaf carotenoid contents was recorded in the control of *Nifa Gold*, 3 hours priming duration while for the same minimum mean value was found to be 0.01 recorded in 10 mM picric acid treatment level of cultivar *Nifa Gold* in 9 hours priming duration (Table 2).

Picric acid treatment levels decreased leaf carotenoid contents of canola plants. According to the Mezzoug *et al.* (2006) and Mostafa (2015), the secondary metabolites of the plants have antimutagenic activities, which is capable of lowering the frequency of mutation by diverse mechanisms of action. They have the capacity to scavenge mutagens or free radicals. Thus, increasing the photochemical composition after mutagens treatments may be due to reduce the deleterious effects of oxidative stress as a natural mechanism in plants after abiotic stresses. But in the present study, decrease in leaf carotenoid contents of canola plants by picric acid was observed which declare picric acid as a counteracting agent against the antimutagenic activities of these secondary metabolites. The decrease in leaf carotenoid contents may be attributed to many reasons. Firstly, it is possible that picric acid treatment caused lethal mutation in the carotenoid transcribing genes, resulting in low production of leaf carotenoids (Shrivastava and Tiwari 2008; Jafri *et al.*, 2011). Secondly, it may also be possible that picric acid is hazardous to the polymerase enzymes itself or its co-factors (sigma and rho factors), thereby reducing the transcribing activity of polymerase enzyme, resulting in low production of messenger RNA. Production of lower amount of messenger RNA effected leaf carotenoid contents (Ananthaswamy *et al.*, 1971). Thirdly, it is also possible that picric acid itself has affected the messenger RNA, resulting in lower quantity of carotenoid contents (Abdel *et al.*, 2012).

Leaf carotenoid contents of plants raised from seed pre-soaked in picric acid solution for higher priming durations (6 hours and 9 hours) were more than that of 3 hours duration. The positive effects of seed priming with picric acid solution may be due the fact that seed embryos got the necessary resistance or adaptability to picric acid with prior soaking that latter on helped the plants in picric acid treatment levels in good production of leaf carotenoid contents. Similarly, picric acid treatment has more positive effects on cultivar *Dur e Nifa* for leaf carotenoid contents than other cultivars.

Effect on leaf phenol contents (mg/ml)

ANOVA for treatment levels (A), canola cultivars (C) and all the four interactions (A×B, B×C, A×C, A×B×C) exhibited significant differences for leaf phenol contents. While non-significant differences were shown by priming durations for phenol contents (Table 1).

Among treatment levels, maximum mean value (0.227) for leaf phenol contents was recorded in control which was higher and significantly different from 5 mM (0.151) and 10 mM (0.191) treatment levels and the rest of two picric acid treatment levels (15 mM and 20 mM) showed non-significant but lower mean values of 0.215 and 0.221 than control respectively.

Plants raised from seeds primed with picric acid for 6 hours showed maximum non-significant mean value of 0.210 which was higher than mean values recorded from 3 hours (0.201) and 9 hours (0.192) priming durations.

Among various canola cultivars highest mean value for leaf phenol contents was recorded for *Nifa Gold* (0.316) followed by *Dur e Nifa* (0.218) and *Abasin-95* (0.068) respectively. Differences among canola cultivars means for leaf phenol contents were found to be significant.

In the first significant interaction of treatment levels × priming durations (A×B), plants raised from seeds primed for 9 hours duration showed maximum mean value (0.285) in control for leaf phenol contents. While the minimum mean value (0.118) for leaf phenol contents was recorded in plants raised from seeds primed for 3 hours duration and 5 mM picric acid treatment level. In the second significant interaction of priming durations × canola cultivars (B×C), plants raised from seeds of cultivar *Nifa Gold* primed for 3 hours duration showed maximum mean value (0.367) for leaf phenol contents. On the other hand, lowest mean value (0.022) leaf phenol content was recorded in plants of cultivar *Abasin-95* primed for the duration of 3 hours. In the third significant interaction of treatment levels × canola cultivars (A×C), plants raised from untreated seeds of *Nifa Gold* showed highest mean value of 0.399 for leaf phenol contents.While minimum mean value (0.038) for leaf phenol contents was recorded for cultivar *Abasin-95* in 5 mM treatment level.

In the fourth interaction of treatment levels × priming durations × canola cultivars (A×B×C), plants raised from seeds of cultivar *Nifa Gold* primed for 9 hours duration, showed maximum mean value (0.523) for leaf phenol contents in control while minimum mean value was found to be 0.010 which was recorded for plants raised from seeds of cultivar *Dur e Nifa* primed for 9 hours duration in 10 mM picric acid treatment level (Table 3).

Picric acid treatment levels showed negative effects on leaf phenol contents of canola plants. Since carotenoids and phenols both show antioxidants properties, their genes almost show the same analogous behavior towards picric acid. The decrease in leaf phenol contents with picric acid of canola plants may be seemed to the same facts of lethal mutation in the phenol transcribing genes, or hazardous nature of picric acid towards the messenger RNA or polymerase enzymes or its co-factors (sigma and rho factors) as were studied for leaf carotenoid contents (Ananthaswamy *et al.*, 1971; Jafri *et al.*, 2011; Abdel *et al.*, 2012).

Leaf phenol contents of plants raised from seeds pre-soaked in picric acid solution for 6 hours priming duration was more than the rest of priming durations. The reason behind the positive effects of seed priming with picric acid solution seemed to be the same fact that seed embryos got the necessary resistance or adaptability to picric acid with prior soaking that latter on helped the plants in picric acid treatment levels in good production of leaf phenol contents. Similarly, picric acid treatment has more positive effects on cultivar *Nifa Gold* for leaf phenol contents than other cultivars.

Effect on proline contents (mg/gm)

ANOVA for all the three factors (Treatment levels, priming durations and canola cultivars) and their interactions (A×B, B×C, A×B×C) except treatment × cultivars exhibited significant differences (Table 1).

Non-treated seeds and seeds treated with 10 mM picric acid treatment level showed maximum mean value of 0.341 for leaf proline contents which was significantly different and higher than mean value (0.327) of plants raised from seeds treated with 15 mM picric acid treatment level. Values for proline contents recorded in control and 10 mM picric acid treatment levels were higher and non-significantly different from mean values found in 5 mM and 20 mM picric acid treatment levels.

Plants rose from seeds of canola cultivars primed with picric acid solution for 3 hours and 6 hours durations almost showed the same mean value of 0.358 for proline contents which was higher and significantly different from mean value (0.288) recorded in plants raised from 9 hours duration primed seeds.

Plants of cultivar *Abasin-95* showed maximum mean value of 0.338 for leaf proline contents followed by cultivar *Dur e Nifa* (0.336) and *Nifa Gold* (0.330) respectively.

In first significant interaction of treatment levels × priming durations (A×B), plants raised from canola seeds primed for 6 hours duration, showed maximum

mean value (0.378) in 5 mM picric acid treatment level for proline contents. While plant rose from seed primed with picric acid solution for 9 hours duration showed minimum mean value (0.274) for leaf proline contents in 5 mM picric acid treatment level. In case of second significant interaction between priming levels and canola cultivars (B×C), plants germinated from seeds of cultivar *Dur e Nifa* primed with picric acid solution for 6 hours duration showed highest mean value of 0.367 for proline contents. On the other hand, cultivar *Abasin-95* and cultivar *Nifa Gold* showed the least mean value (0.286) for leaf proline contents in highest priming duration (9 hours). In the fourth interaction of treatment levels × priming durations × canola cultivars (A×B×C), maximum mean value (0.299) for leaf proline contents was recorded in plants raised from seeds of cultivar *Nifa Gold* primed with picric acid solution for 6 hours duration in 10 mM picric acid treatment level. While the least mean value (0.267) for leaf proline contents was shown by plants of cultivar *Abasin-95* primed with picric acid solution for 9 hours duration in untreated seeds (Table 4).

Production of enough leaf proline contents in plants raised from canola seeds sown in picric acid solution treated pots indicated stressful conditions created by the mutagen (Jahangir *et al*., 2012). Plants rose from seeds of canola cultivars primed with picric acid solution for 3 hours and 6 hours durations almost showed the same leaf proline contents indicating the greater efficiency of these priming levels for activating stress combating genes for canola plants over 9 hours priming duration. The phenomenon of proline accumulation is known to occur under stressful (water deficit, salinity, low temperature, heavy metal exposure, mutagens etc.) conditions. Our study regarding effects of picric acid on canola cultivars proved the same earlier mentioned facts explained by other workers for the production of increased proline contents by various plants. Apart from acting as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular membranes and proteins, scavenging free radicals and buffering cellular redox potential under stress conditions (Naidu *et al*., 1991; Bassi and Sharma 1993; Hare and Cress 1997; Hare and Cress 1998; Rhodes *et al*., 2002; Munns 2005; Sharma and Dietz 2006). From the above-mentioned facts, it can be concluded easily, that picric acid can cause osmotic disturbances, create free radicals and can have severe effects on sub cellular structures in plants, as proline contents counteract such stressful consequences to normal conditions.

Effect on leaf protein contents (mg/gm)

ANOVA for treatment levels (A), priming durations (B) and canola cultivars (C) and all the four interactions (A×B, B×C, A×C, A×B×C) were non-significant for protein contents (Table 1 and 5).

Effect on leaf sugar contents (mg/gm)

ANOVA of leaf sugar contents exhibited significant differences for treatment levels (A), priming durations (B), canola cultivars (C) and all the four interactions (A×B, B×C, A×C, A×B×C) (Table 1).

Mean values for leaf sugar contents recorded from plants in 5 mM (0.973) and 10 mM (0.969) picric acid treatment levels exceeded mean value (0.963) shown by control. But the differences among them were non-significant. However, mean values for leaf sugar contents recorded from plants of 15 mM (0.954) and 20 mM (0.960) picric acid treatment levels were lower but non-significantly different from control.

Plants raised from seeds primed for 9 hours durations showed maximum mean value (0.982) leaf sugar contents than 3 hours (0.975) and 6 hours (0.934) priming durations. Differences among plants for leaf sugar contents between 9 hours and 3 hours priming durations were non-significant. However, mean values for sugar contents recorded from these two priming durations exhibited significant differences from 6 hours priming duration.

Cultivar *Abasin-95* showed the maximum mean value for leaf sugar contents which was 0.967 followed by cultivar *Nifa Gold* (0.963) and *Dur e Nifa* (0.961).

In the first interaction between treatment levels and

priming durations (A×B), plant raised from seeds primed with picric acid for 9 hours duration showed the maximum mean value of 0.998 in 5 mM picric acid treatment level. While the mean value of 0.921 for leaf sugar contents was proved to be the least, recorded from seeds primed with picric acid solution for 6 hours duration in 20 mM picric acid treatment level. In the second interaction of priming durations × canola cultivars (B×C), cultivar *Dur e Nifa* showed maximum mean value (0.930) for leaf sugar contents in 6 hours priming duration. In the fourth interaction of treatment levels × priming durations × canola cultivars (A×B×C), plants raised from seeds of cultivar *Abasin-95*, primed with picric acid solution for 3 hours duration showed maximum interaction mean value (1.00) for leaf sugar contents while 0.900 was the least mean interaction value for the same parameter, recorded for cultivar *Dur e Nifa*, primed with picric acid solution for 6 hour duration in 20 mM picric acid treatment level (Table 6).

Production of enough leaf sugar contents in plants raised from canola seeds sown in picric acid solution treated pots indicated stressful conditions created by the mutagen (Jahangir *et al.*, 2012). Plants rose from seeds of canola cultivars primed with picric acid solution for 9 hours and 3 hours durations almost showed the same leaf sugar contents indicating the greater efficiency of these priming levels for creating stressful conditions for plants over 6 hours priming duration. The phenomenon of soluble sugars accumulation is known to occur under stressful (water deficit, salinity, low temperature, heavy metal exposure, mutagens etc.) conditions. So, it can be concluded easily, that picric acid can cause osmotic disturbances, create free radicals and can have severe effects on sub cellular structures in plants, as sugar contents counteract such stressful consequences to normal conditions as proline contents do.

Conclusion

From the present study number of clear conclusions can be drawn which are stated in numbers. First lower doses of picric acid (5 mM, 10 mM) are stimulatory for leaf proline and sugar contents of canola plants. Secondly, the applied doses of picric acid are inhibitory for leaf carotenoid and phenol contents of canola plants. Thirdly, seed priming has positive effects on the canola plants grown in picric acid regimes. The 4th conclusion is regarding priming durations, canola plants raised from seeds primed with picric acid solution for 6 hours duration proved good for leaf carotenoid, phenol and proline contents while for leaf sugar contents priming duration of 9 hours was found good. The last conclusion is about different canola accessions of which cultivar *Abasin-95* superseded other cultivars in leaf proline and sugar contents. While cultivar *Dur e Nifa* and *Nifa Gold* were found to produce high leaf carotenoid and phenol contents.

Recommendation

For further studies on the same chemical and same plant, lower doses of picric acid (<10 mM) and priming durations (<6 hours) are recommended for good and pronounced results.

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