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Smoke produced from plants waste material elicits growth of wheat (*Triticum aestivum* L.) by improving morphological, physiological and biochemical activity

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ABSTRACT

The experimental work presented in this study was carried out with the hypothesis that plant derived smoke enhanced the morphological, physiological and biochemical attributes of a cereal crop, wheat (*Triticum aestivum* L.). Furthermore, this study supported the hypothesis that plant derived smoke acts as vegetative growth promoter, inexpensive, rapid and most appropriate eco-friendly bio-fertilizer for sustainable agriculture. Plant derived smoke was generated by burning of plant material (leaf, straws etc) in a specially designed furnace, and seeds were treated with this smoke for different time duration. Four level of plant derived smoke (1 h, 2 h, 3 h and 4 h) along with control were tested on four wheat cultivars in CRD repeated pot experiment. The smoke-related treatments modified number of morphological, physiological and biochemical features of wheat. Compared with the control, aerosol smoke treatment of the seeds significantly improved root length (2.6%), shoot length (7.7%), RFW (0.04%), SFW (0.7%), SDW (0.1%) and leaf area (63.9%). All the smoke-related treatments significantly promoted RWC (17.3%), water potential (1.5%), osmotic potential (1.4%) and MSI (14.6%) whereas a pronounced increase in chlorophyll a (24.9%), chlorophyll *b* (21.7%) and total chlorophyll contents (15.5%) were recorded in response to aerosol-smoke treatments. Plant derived smoke exposure applied for short time i.e. 1 h & 2 h induced significant results as compared to prolonged PDS exposure (3 h and 4 h). The best results were observed in Pak-13 and Glaxy-13 wheat cultivars. These findings indicated that the plant-derived smoke treatment has a great potential to improve morphological, physiological and biochemical features of wheat crop.

1. Introduction

Wheat (*Triticum aestivum* L.) is an annual, self-pollinated, long-day cereal crop, and is one of the most important food item in Pakistan as well as in the world. It is also the most cultivated and domesticated grass worldwide. Due to its dietary importance it is considered 2nd big source of calories and major source of proteins in human diet in developing countries [1]. Wheat covers around maximum part of nutritional requirements of people by adding up one half of the calories in human's food. Every nation of the world utilizes about 600 million metric tons of wheat per annum for commercial purpose [2,3]. According to estimation of International Food Policy Research Institute (IFPRI) due to the rapidly growing population of the world, consumption of wheat will climb up from 552 to 775 million tons by year 2020, ultimately results consumption up to 60% by the year 2050 [4,5].

There is an intensive need to increase wheat production to meet the rapidly increasing population demands. The constant and never-ending research efforts has always been performed to boost up wheat production and its yield per unit area [6] but yet there is substantial need of perfection, particularly to intensify hard work for getting sustained wheat yield to fulfill the ultimate demands of ever increasing population. Conventional plant breeding methods and agricultural practices although have been practiced successfully since 1960s for the production of improved wheat varieties and to obtain more yield but have limited potential to meet such a great challenge due to availability of

Abbreviations: PDS, plant derived smoke; RFW, root fresh weight; SFW, shoot fresh weight; SDW, shoot dry weight; RWC, relative water contents; MSI, membrane stability index; SL, strigolactone

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Table 1
Effect of plant derived smoke with different treatments on morphological attributes of wheat (*Triticum aestivum* L.).

Treatments	Varieties	Root Length (cm)	Shoot Length (cm)	Root Fresh Weight (g)	Shoot Fresh Weight (g)	Root Dry Weight (g)	Shoot Dry Weight (g)	Leaf Area (cm^2)
To	V1	7.4 ± 2.0^{ab}	36.1 ± 1.0^{a}	0.07 ± 0.0^{df}	0.5 ± 0.1^{c}	0.02 ± 0.0^{bd}	0.13 ± 0.0^{ef}	246.9 ± 0.9°
To	V2	7.2 ± 3.1^{ad}	42.3 ± 1.3^{fg}	0.09 ± 0.0^{de}	0.7 ± 0.4^{bc}	0.01 ± 0.1^{cd}	0.15 ± 0.0^{cd}	251.5 ± 0.5^{n}
To	V3	7.7 ± 1.9^{ce}	45.2 ± 1.1^{c}	0.10 ± 0.0^{bd}	1.1 ± 0.0^{ac}	0.03 ± 0.0^{bc}	0.17 ± 0.0^{bc}	263.8 ± 0.7^{l}
To	V4	6.7 ± 1.0^{de}	41.2 ± 1.1^{h}	0.11 ± 0.1^{a}	1.1 ± 0.3^{ac}	0.04 ± 0.0^{ab}	0.20 ± 0.1^{bc}	281.8 ± 0.7^{hi}
T1	V1	3.7 ± 2.8^{f}	43.2 ± 1.0^{ef}	0.06 ± 0.0^{ef}	0.5 ± 0.1^{c}	0.05 ± 0.1^{a}	0.10 ± 0.0^{g}	160.2 ± 0.8^{q}
T1	V2	9.7 ± 3.4^{bc}	47.1 ± 1.1^{b}	0.10 ± 0.0^{cd}	1.2 ± 0.3^{ac}	0.03 ± 0.0^{cb}	0.21 ± 0.1^{bd}	268.3 ± 0.6^{k}
T1	V3	5.8 ± 0.8^{ef}	51.3 ± 1.4^{a}	0.10 ± 0.1^{de}	1.5 ± 0.5^{ab}	0.02 ± 0.0^{ad}	0.24 ± 0.1^{ab}	305.9 ± 0.9^{c}
T1	V4	6.3 ± 1.2^{ef}	52.9 ± 0.2^{a}	0.12 ± 0.1^{bc}	1.4 ± 0.3^{ab}	0.03 ± 0.0^{bc}	0.25 ± 0.1^{ab}	296.8 ± 0.8^{f}
T2	V1	10.3 ± 2.6^{a}	41.4 ± 1.3^{gh}	0.08 ± 0.0^{cd}	0.9 ± 0.1^{bc}	0.02 ± 0.0^{bc}	0.13 ± 0.1^{de}	203.2 ± 0.3^{p}
T2	V2	6.0 ± 1.5^{ef}	47.9 ± 0.9^{b}	0.09 ± 0.0^{ce}	1.0 ± 0.3^{b}	0.01 ± 0.1^{cd}	0.20 ± 0.0^{de}	282.5 ± 0.5^{h}
T2	V3	7.2 ± 2.6^{bc}	47.1 ± 1.0^{b}	0.13 ± 0.0^{bc}	1.5 ± 0.8^{ab}	0.02 ± 0.1^{cd}	0.25 ± 0.1^{ab}	335.9 ± 0.8^{b}
T2	V4	6.9 ± 2.3^{bd}	51.8 ± 1.6^{a}	0.07 ± 0.1^{de}	1.4 ± 0.6^{ab}	0.01 ± 0.1^{cd}	0.18 ± 0.1^{cd}	302.7 ± 0.6^{d}
T3	V1	8.2 ± 2.3^{ac}	38.3 ± 1.7^{i}	0.08 ± 0.0^{de}	0.9 ± 0.3^{bc}	0.02 ± 0.0^{bc}	0.13 ± 0.0^{de}	261.7 ± 1.5^{m}
T3	V2	9.6 ± 1.4^{cd}	44.4 ± 0.5^{cd}	0.05 ± 0.0^{f}	0.8 ± 0.1^{bc}	0.01 ± 0.0^{d}	0.11 ± 0.0^{fg}	277.9 ± 0.9^{j}
T3	V3	10.1 ± 1.4^{ab}	45.0 ± 1.0^{cd}	0.20 ± 0.0^{a}	1.8 ± 0.9^{a}	0.05 ± 0.0^{ab}	0.33 ± 0.1^{a}	345.7 ± 0.6^{a}
T3	V4	6.7 ± 0.3^{c}	48.1 ± 1.0^{b}	0.09 ± 0.1^{cf}	1.1 ± 0.4^{ac}	0.02 ± 0.0^{bd}	0.17 ± 0.0^{bc}	300.2 ± 1.4^{e}
T4	V1	9.7 ± 2.9^{bc}	36.1 ± 1.0^{j}	0.10 ± 0.0^{ce}	1.1 ± 0.5^{ab}	0.02 ± 0.0^{bc}	0.17 ± 0.1^{bc}	261.9 ± 0.9^{m}
T4	V2	6.5 ± 1.0^{de}	43.4 ± 0.6^{de}	0.07 ± 0.1^{df}	0.8 ± 0.2^{bc}	0.01 ± 0.0^{d}	0.13 ± 0.0^{de}	263.5 ± 0.4^{l}
T4	V3	7.5 ± 1.8^{cd}	41.7 ± 0.6^{fg}	0.11 ± 0.1^{ce}	1.2 ± 0.6^{bc}	0.03 ± 0.0^{bd}	0.28 ± 0.1^{ab}	290.8 ± 0.7^{g}
T4	V4	6.3 ± 0.6^{df}	45.0 ± 1.0^{cd}	0.13 ± 0.1^{bc}	1.4 ± 1.1^{ab}	0.03 ± 0.0^{ac}	0.26 ± 0.2^{ab}	280.5 ± 0.5^{i}
LSD		1.647	0.872	0.028	0.401	0.016	0.058	0.649

Notes: Results are denoted as mean ± SE. Different letters in columns indicate statistically significant treatment effect based on Duncan's Multiple Range Test at 5% ($p < 0.05$) probability.
To = Control; T1 = 1 h treatment of PDS; T2 = 2 h treatment of PDS; T3 = 3 h treatment of PDS; T4 = 4 h treatment of PDS.

limited gene pool and lack of application of advanced agricultural techniques [7,[6]]. The process of germination and plant growth is a complex process and can be affected at different stages by many factors and interactions of factors such as temperature, water availability, oxygen, light, substrate, maturity of seed and physiological age of seed.

In this context, great potential is embodied in discovery of plant derived smoke for promoting seed germination and enhancing plant growth [8]. Plant-derived smoke play a positive role in enhancing seed germination and plant growth of many hard-to-germinate and rare and threatened species [7,9]. Plant derived smoke has also been shown enhancing seed germination, seedling growth and vigor of a wide range of agricultural and horticultural crops [10,[8]]. One pre-requisite for the application of plant derived smoke for enhancing wheat plant growth is the use of smoke, purely obtained from burning of plant material, as previous studies suggested that smoke obtained from burning of plant material has significantly increase the yield components of different vegetables and crops [9,11]. This technology is a good substitute to traditional agricultural practices applied to enhance seed germination and plant growth in different plants and crops as it is cheap, easily approachable and of more useful method to obtained high yield. However, as yet no comprehensive studies have been conducted on wheat to substantiate the net effect and possible role of plant-derived smoke treatments. Thus, in an attempt to boost cereal crop production the current study was executed to assess the effects of plant derived smoke on certain important morphological, physiological and biochemical features of wheat. The specific objectives were to examine the effects of various smoke applications (aerosol-smoke treatments) on morphological, physiological and biochemical growth of selected wheat varieties grown under greenhouse conditions.

2. Materials and methods

2.1. Experimental

This research was conducted to check the growth, physio-morphic and biochemical expression of wheat in response to plant derived smoke. Plant material such as leaves of *Pongamia glabra*, *Populus deltoids*, *Morus alba* and straws of *Oryza stiva* and *Cynodon dactylon* were collected from the outskirts of PMAS-AAUR Rawalpindi, Pakistan. Plant derived aerosol smoke was produced from burning of semi dried plant material in a specially designed furnace and aerosol smoke was collected in jars for treatments. Seeds of four different wheat (*Triticum aestivum* L.) varieties i.e. NARC-2011, Aas-2011, Pakistan-2013 and Glaxy-2013 were obtained from National Agriculture Research Institute (NARC) Islamabad, Pakistan. Surface sterilization of seeds was done by dipping in 10% sodium hypochlorite solution for 10 min and then washed carefully with distilled water [8]. After sterilization, seeds of wheat varieties were treated with cool plant derived aerosol smoke for 1 h, 2 h, 3 h and 4 h time duration to check the possible outcomes of smoke treatments on wheat. The pot experiment was carried out in a green house maintained at temperature 23 ± 2.5 °C, relative humidity between 25% and 52% and a mid-day photosynthetic photon flux density of 405 ± 7.5 $\mu mol\, m^{-2}\, s^{-1}$. The wheat seeds were directly sown in earthen pots (250 mm in diameter and 210 mm in height) filled with sandy loam soil (sand: 52.5%, silt: 2.5% and clay: 45%). All plants grew in the specified soil media and no fertilizer was applied during the entire growing season. At first, 10 seeds per pot were sown, afterwards thinned to a six plants per pot at trifoliate stage. The experiment comprised the following five treatments: To (control), T1 (seeds treated with PDS for 1 h), T2 (seeds treated with PDS for 2 h), T3 (seeds treated with PDS for 3 h), T4 (seeds treated with PDS for 4 h) with four varieties and each treatment was repeated three times giving a total of 60 pots. After applying the plant derived smoke, all the seeded pots were irrigated with little, approximately 500 ml, tap water to initiate germination. Afterwards, the pots were randomly placed on a 1-m-high greenhouse bench and were manually irrigated with tap water to field capacity every third day. Within the greenhouse, the pots were randomly rotated on weekly basis to minimize positional effects. The whole experiment was conducted from the beginning of November 2015 till March 2016.

2.2. Data collection for selected morphological, physiological and biochemical parameters

During the course of growth several morphological, physiological and biochemical parameters were recorded after 90 days through

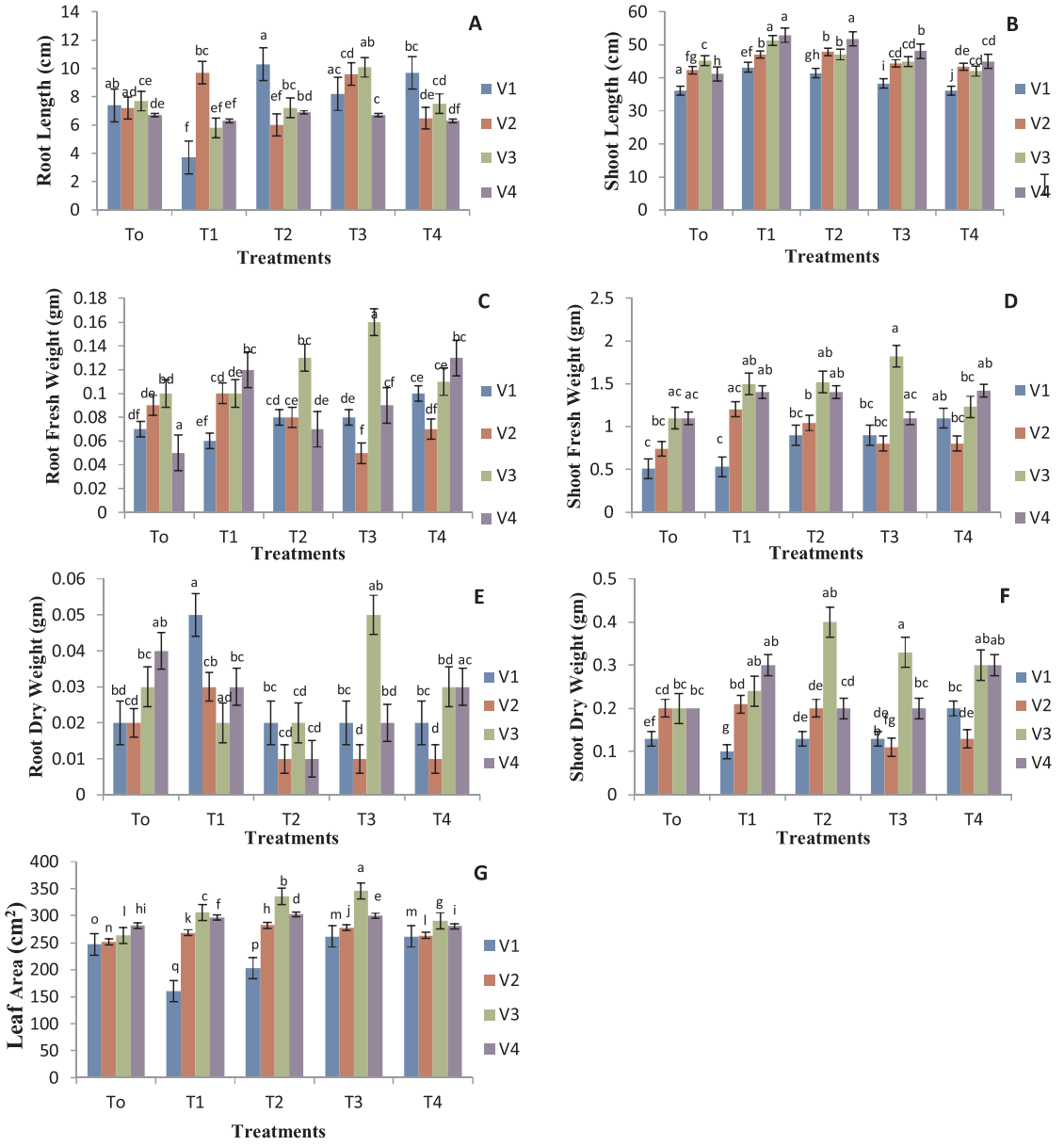

Fig. 1. (A,B,C,D,E,F,G). Curves of selected morphological parameters showing various patterns in response to plant derived smoke treatments among wheat varieties. To = Control; T1 = 1 h treatment of PDS; T2 = 2 h treatment of PDS; T3 = 3 h treatment of PDS; T4 = 4 h treatment of PDS; V1 = NARC-11; V2 = Aas-11; V3 = Pak-13; V4 = Glaxy-13.

random sampling method. These parameters included plant height (root and shoot length), leaf area, fresh and dry biomass, relative water contents, membrane stability index, water potential, osmotic potential, chlorophyll contents and estimation of biochemical parameters such as proline, free amino acids and soluble sugar contents.

2.3. Measurements of morphological parameters

Three plants from each treatment were taken for measuring morphological parameters. Root and shoot length was measured with the help of 1-m power-tap. After taking fresh weight of each sample, dry biomass weight was recorded individually after oven drying at 65 °C. Leaf area was measured by using leaf area meter CID, CI-202.

2.4. Determination of physiological parameters

2.4.1. Relative water contents

Relative water contents (RWC) was analyzed according to method given by Unyayer et al. [12] method. The fresh weight of flag leaf from each treatment was measured. These leaves were then engrossed in mineral water in 100 ml beakers and left there till the saturation point for a day. After that entirely turgid leaves had been weighed for saturated mass. Then same leaves were dehydrated in oven for 72 h at 72 °C until invariable weight of these observing leaves was obtained.

$$RWC = [(\text{fresh weight- dry mass})/(\text{saturated mass} - \text{dry mass})] \times 100$$

2.4.2. Leaf osmotic potential

Cell sap of flag leave was obtained and the osmotic potential was measured with an osmometer by following method of Capell and Doerffling [13]. Cell sap (50 µl) was obtained by enclosing the leaf material in a plastic disposable syringe and stored at freezing temperature. Later it was pushed to pour out the sap from the mitigated leaf. Evaluations were taken from osmometer in mosmol/kg and transformed to MPa by using following formula:

$$\text{Osmotic potential} = \text{osmolality (mosmol)} \times 0.831 \times 10 - 5 \times T(K)$$

Table 2
Effect of plant derived smoke with different treatments on physiological attributes of wheat (*Triticum aestivum* L.).

Treatments	Varieties	Relative Water Contents (%)	Water Potential (MPa)	Osmotic Potential (MPa)	MSI (%)	Chlo a (ug/ml)	Chlo b (ug/ml)	Total Chlo Contents (ug/ml)
To	V1	32.3 ± 1.2^{cd}	0.6 ± 0.1^{f}	0.5 ± 0.0^{ij}	67.7 ± 2.5^{k}	12.7 ± 1.5^{k}	9.3 ± 0.3^{gh}	17.4 ± 1.4^{k}
To	V2	53.7 ± 3.2^{de}	0.3 ± 0.1^{h}	0.4 ± 0.0^{j}	71.7 ± 1.5^{ij}	8.5 ± 0.4^{l}	3.7 ± 0.1^{k}	13.0 ± 0.2^{l}
To	V3	56.0 ± 1.7^{de}	0.8 ± 0.1^{ef}	0.7 ± 0.1^{gh}	73.7 ± 2.1^{hi}	10.9 ± 0.2^{kl}	8.1 ± 0.1^{ij}	20.5 ± 0.5^{j}
To	V4	40.7 ± 6.6^{ab}	0.6 ± 0.1^{f}	0.7 ± 0.0^{hi}	68.7 ± 3.1^{jk}	13.3 ± 0.4^{k}	7.3 ± 0.1^{j}	21.4 ± 0.4^{ij}
T1	V1	45.7 ± 9.1^{ab}	0.8 ± 0.1^{ef}	0.9 ± 0.0^{fg}	71.3 ± 3.1^{ij}	21.1 ± 2.0^{i}	10.3 ± 0.6^{gh}	20.6 ± 1.8^{j}
T1	V2	35.7 ± 7.3^{cd}	1.2 ± 0.2^{d}	1.2 ± 0.2^{de}	76.0 ± 2.0^{fg}	11.3 ± 1.5^{k}	8.5 ± 0.8^{hi}	15.4 ± 0.6^{k}
T1	V3	67.0 ± 6.9^{bc}	2.3 ± 0.3^{a}	2.1 ± 0.1^{a}	78.0 ± 2.6^{de}	19.0 ± 2.0^{ij}	14.0 ± 2.1^{f}	27.3 ± 1.2^{fg}
T1	V4	50.7 ± 2.0^{de}	2.1 ± 0.2	1.9 ± 0.0^{a}	74.7 ± 3.1^{gh}	26.8 ± 0.2^{gh}	11.2 ± 0.2^{g}	23.3 ± 0.6^{hi}
T2	V1	61.0 ± 7.3^{bc}	1.6 ± 0.1	1.5 ± 0.2^{bc}	77.3 ± 2.1^{de}	26.7 ± 1.5^{gh}	14.3 ± 1.2^{f}	25.3 ± 1.1^{gh}
T2	V2	42.3 ± 6.4^{ab}	0.7 ± 0.1^{f}	0.8 ± 0.0^{fg}	79.7 ± 2.1^{cd}	17.3 ± ± 2.5^{j}	10.8 ± 1.1^{gh}	19.9 ± 0.3^{j}
T2	V3	73.3 ± 7.0^{a}	1.7 ± 0.1	1.7 ± 0.2^{b}	86.0 ± 3.0^{ab}	28.3 ± 1.5^{fg}	18.6 ± 1.3^{de}	30.6 ± 2.2^{de}
T2	V4	70.3 ± 1.0^{ab}	2.0 ± 0.1	1.6 ± 0.2^{b}	78.0 ± 2.6^{de}	31.8 ± 2.6^{de}	18.6 ± 1.6^{ef}	28.7 ± 1.8^{ef}
T3	V1	50.7 ± 2.5^{de}	1.2 ± 0.2	1.4 ± 0.2^{cd}	82.3 ± 2.1^{bc}	30.3 ± 1.5^{ef}	20.1 ± 2.0^{d}	27.7 ± 1.2^{f}
T3	V2	64.3 ± 1.5^{bc}	0.5 ± 0.1^{gh}	0.7 ± 0.1^{gh}	77.0 ± 2.6^{ef}	24.3 ± 1.5^{h}	14.2 ± 2.2^{f}	22.0 ± 1.6^{ij}
T3	V3	51.3 ± 2.0^{de}	1.0 ± 0.1^{de}	1.1 ± 0.2^{e}	88.3 ± 1.5^{a}	34.8 ± 1.6^{bc}	25.5 ± 2.3^{bc}	33.6 ± 0.9^{bc}
T3	V4	39.7 ± 9.2^{ab}	1.6 ± 0.3^{c}	1.2 ± 0.2^{de}	81.0 ± 2.0^{cd}	34.0 ± 1.0^{cd}	18.8 ± 1.9^{de}	32.3 ± 0.9^{cd}
T4	V1	22.0 ± 9.5^{f}	1.1 ± 0.0	0.9 ± 0.0^{f}	80.0 ± 1.0^{cd}	35.3 ± 1.5^{bc}	26.3 ± 2.7^{b}	32.1 ± 1.9^{cd}
T4	V2	72.7 ± 2.5^{a}	0.6 ± 0.1^{f}	0.6 ± 0.0^{i}	76.0 ± 2.6^{fg}	28.3 ± 2.1^{fg}	18.3 ± 1.6^{de}	25.3 ± 1.3^{gh}
T4	V3	28.3 ± 9.5^{ef}	0.8 ± 0.1^{ef}	0.9 ± 0.0^{fg}	78.7 ± 1.5^{cd}	38.2 ± 2.1^{a}	31.0 ± 2.7^{a}	36.9 ± 2.0^{a}
T4	V4	32.0 ± 7.1^{ef}	1.2 ± 0.0^{d}	0.7 ± 0.1^{gh}	68.7 ± 1.0^{jk}	36.7 ± 1.6^{ab}	22.9 ± 1.6^{c}	34.7 ± 1.2^{b}
LSD		15.668	0.118	0.101	1.888	1.32	1.285	1.048

Notes: Results are denoted as mean ± SE. Different letters in columns indicate statistically significant treatment effect based on Duncan's Multiple Range Test at 5% ($p < 0.05$) probability.
To = Control; T1 = 1 h treatment of PDS; T2 = 2 h treatment of PDS; T3 = 3 h treatment of PDS; T4 = 4 h treatment of PDS

2.4.3. Water potential

Leaf water potential was recorded by using an apparatus known as Scholander pressure chamber [14]. Flag leaves were cut out from the required plant and were fixed in specimen holders over pressure vessel of Scholander pressure chamber. The pressure was build within the pressure vessel till the appearance of sap at the cutting end of the leaf. Reading of the pressure appeared is equal to the negative strength with which the water in plant is held. It was measured and calculated in MPa.

2.4.4. Membrane stability index (MSI)

Membrane stability index (MSI) was measured by following method proposed by Sairam [15]. Firstly the leaf disc (100 mg) were washed with water thereafter double distilled water was used for washing, then discs were heated with double distilled water (100 ml) in water bath at 40 °C for half an hour. Then first electrical conductivity (C1) was verified by using EC meter. Afterward, the same experimental procedure was repeated at 100 °C temperature for 10 min and their electrical conductivity (C2) was recorded. MSI was measured with the help of following formula:

$$\text{Membrane stability index (MSI)} = \{1\text{-}(C1/C2)\} \times 100$$

2.4.5. Leaf chlorophyll contents

Determination of photosynthetic pigments was carried out when plants were two month old, as aging causes rapid decline in pigments concentration. Quantification of the photosynthetic pigments, chlorophyll a, b and total chlorophyll contents, were performed following the protocols described by Bruinsma [16]. The 0.2gfresh leaf samples (the third leaf from the shoot-tip) were destructively sampled from each of the five treatments and homogenized using a mortar and pestle in 10mLice-cold acetone. Thereafter, the solution was filtered through Whatman No. 1 filter paper at room temperature. The absorbance of the resultant filtrate was measured at three wavelengths (i.e. 645, 652 and 663 nm) with three technical replicates. The pigment content was estimated using following formulae; values were calculated as micrograms per gram fresh weight:

$$\text{Chlorophyll a contents} = 12.7(A_{663}) - 2.7(A_{645})$$

$$\text{Chlorophyll b contents} = 22.9(A_{645}) - 4.7(A_{663})$$

$$\text{Total chlorophyll contents} = (A_{652} \times 1000/34.5)$$

2.5. Estimation of biochemical parameters

2.5.1. Proline contents

Proline contents were estimated by the process of Bates et al. [17] through use of spectrophotometer. For this material of the wheat plant (0.5 g) was homogenized with 4 ml sulfosalicylic acid (0.3%) in mortar and stored whole night at 5 °C. Solution was centrifuged at 3000 rpm for 5 min at room temperature. Supernatant was mixed up with 4 ml acidic ninhydrin reagent. Obtained material was mechanically shaken; then these tubes were warmed in the water bath for an hour. After that substance in the tube was cooled and the solution was obtained with 4 ml of toluene. The transmittance of toluene layer was traced at 520 nm. The absorption of the indefinite sample was observed with the help of standard curve.

$$\text{Proline (µg/ml)} = \text{Sample absorbance} \times \text{dilution factor} \times \text{K value/fresh weight of plant tissue}$$

2.5.2. Free amino acids

Free amino acids of each sample were estimated by using ninhydrin method [18]. A leaf extract was prepared after grinding 0.2 g of fresh leaf material in sodium phosphate buffer. After filtration 1 ml was taken from this filtrate in separate test tubes, then 1 ml of pyridine solution (10%) and 1 ml of ninhydrin solution (2%) were mixed in it and were placed in water bath for 30 min. Afterward this mixture was diluted to requisite concentration and absorbance was noticed at 570 nm. Following formula was applied to calculate free amino acids (mg/g fresh weight):

$$\text{Total free amino acids} = \text{Reading of sample} \times \text{sample volume} \times \text{dilution factor/weight of fresh plant tissue} \times 1000$$

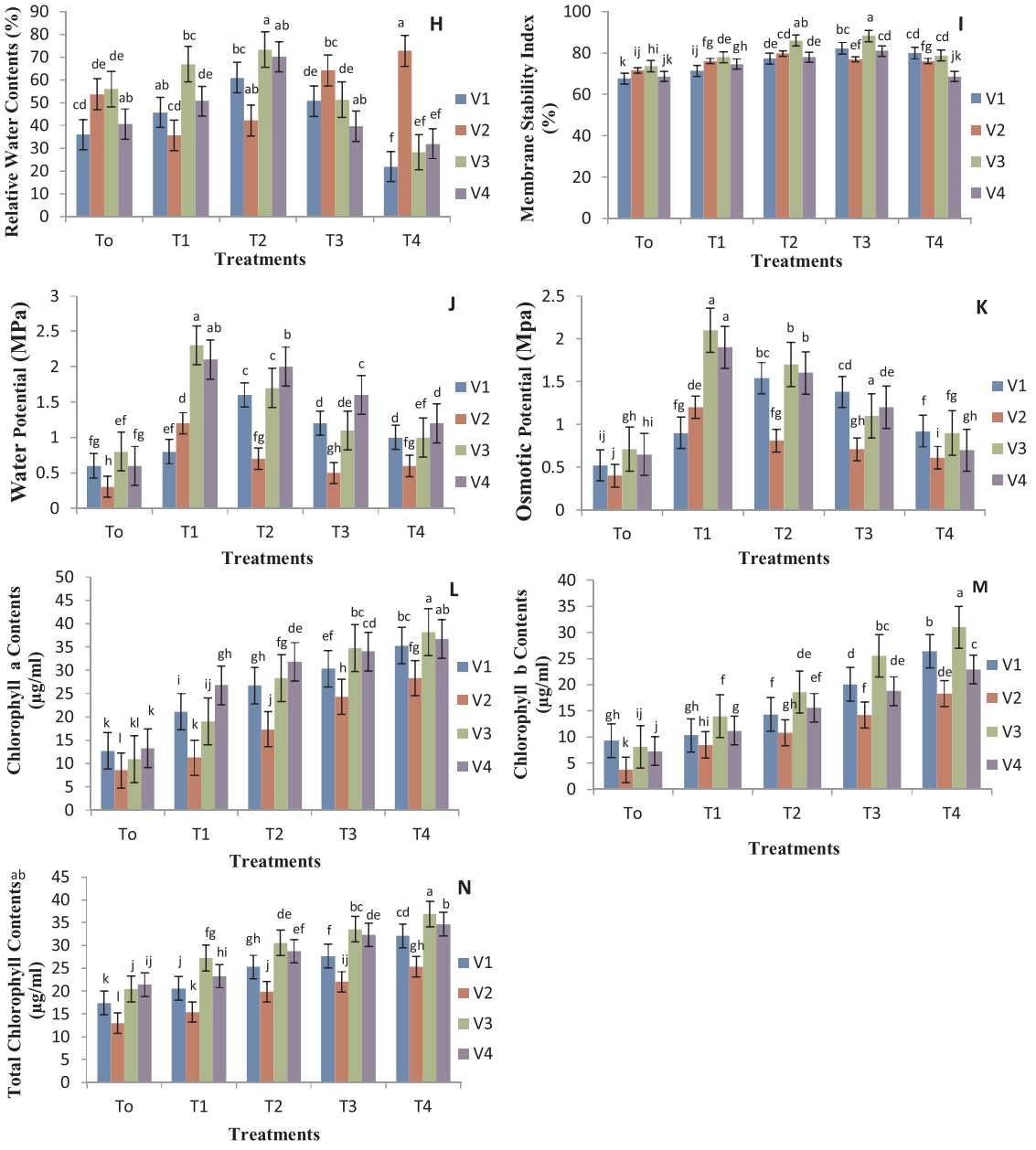

Fig. 2. (H,I,J,K,L,M,N). Curves of selected physiological parameters showing various patterns in response to plant derived smoke treatments among wheat varieties. To = Control; T1 = 1 h treatment of PDS; T2 = 2 h treatment of PDS; T3 = 3 h treatment of PDS; T4 = 4 h treatment of PDS; V1 = NARC-11; V2 = Aas-11; V3 = Pak-13; V4 = Glaxy-13.

2.5.3. Sugar analysis

Sugar analysis was recorded with the help of phenol sulphuric acid method [19]. Ground material of the plant (0.5 g) was added to the test tubes after adding 10 ml of 80% ethanol. This whole mixture was then placed in boiling water bath for an hour at 80 °C temperature. 0.5 ml of this extract was taken in additional test tubes, 0.5 ml of unionized water and 1 ml of 18% phenol was mixed into it, these test tubes were allowed to set for an hour at 25 °C and then readings were observed with spectrophotometer at 490 nm. Total sugar content was calculated by the following formula:

Sugar (µg/ml) = Sample absorbance × dilution factor × K value/ weight of fresh plant tissue

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was conducted and means of the treatments were compared using Duncan's multiple range test at 5% level of significance. GenStat 8th Edition statistical package release statistics 8.1 software was used for statistical analysis.

3. Results

Results of the one-way ANOVA conducted to detect the effect of the smoke related treatments on morphological performances of wheat are provided in Table 1. Compared with the control, aerosol-smoke treatments significantly ($p \leq 0.05$) improved plant root shoot length, fresh and dry weight and leaf area of wheat plants. In comparison with the control 1 h aerosol smoke treatment resulted increase in root length (2.5%), shoot length (11.7%), SFW (1.4%), SDW (0.5%) and in leaf area (42.9%) respectively. In 2 h PDS treatment significant increase was

Table 3
Effect of plant derived smoke with different treatments on biochemical attributes of wheat (*Triticum aestivum* L.).

Treatments	Varieties	Proline Contents (ug/ml)	Soluble Sugar (mg/g)	Free Amino Acids (mg/g)
To	V1	10.1 ± 0.3^{a}	32.0 ± 0.2^{b}	8.9 ± 0.2^{a}
To	V2	9.2 ± 0.4^{b}	29.1 ± 0.5^{d}	9.2 ± 0.3^{a}
To	V3	10.3 ± 0.4^{a}	31.9 ± 0.3^{b}	8.0 ± 0.1^{b}
To	V4	9.2 ± 0.6^{b}	33.1 ± 0.3^{a}	7.2 ± 0.5^{c}
T1	V1	2.3 ± 0.4^{j}	24.5 ± 0.5^{h}	2.8 ± 0.2^{g}
T1	V2	3.4 ± 0.5^{hi}	24.1 ± 0.3^{h}	3.3 ± 0.4^{g}
T1	V3	3.3 ± 0.3^{i}	26.1 ± 0.3^{g}	4.0 ± 0.3^{f}
T1	V4	4.3 ± 0.4^{g}	24.2 ± 0.5^{h}	6.1 ± 0.1^{d}
T2	V1	3.9 ± 0.2^{gh}	26.1 ± 0.4^{g}	3.9 ± 0.2^{f}
T2	V2	6.1 ± 0.4^{e}	26.2 ± 0.3^{g}	6.1 ± 0.3^{d}
T2	V3	5.1 ± 0.3^{f}	28.1 ± 0.4^{e}	5.1 ± 0.3^{e}
T2	V4	5.3 ± 0.4^{f}	27.0 ± 0.1^{f}	6.1 ± 0.3^{d}
T3	V1	5.0 ± 0.3^{f}	27.0 ± 0.4^{f}	4.9 ± 0.3^{e}
T3	V2	7.3 ± 0.6^{d}	27.9 ± 0.4^{e}	7.0 ± 0.2^{c}
T3	V3	6.2 ± 0.3^{e}	29.0 ± 0.4^{d}	6.2 ± 0.4^{d}
T3	V4	6.3 ± 0.4^{e}	28.2 ± 0.2^{e}	6.4 ± 0.5^{d}
T4	V1	7.2 ± 0.6^{d}	29.1 ± 0.6^{d}	7.4 ± 0.5^{c}
T4	V2	8.1 ± 0.4^{c}	28.2 ± 0.7^{e}	8.8 ± 0.3^{a}
T4	V3	7.0 ± 0.1^{d}	30.2 ± 0.7^{c}	7.9 ± 0.2^{b}
T4	V4	8.1 ± 0.3^{c}	29.2 ± 0.5^{d}	7.1 ± 0.3^{c}
LSD		0.313	0.336	0.249

Notes: Results are denoted as mean ± SE. Different letters in columns indicate statistically significant treatment effect based on Duncan's Multiple Range Test at 5% ($p < 0.05$) probability.
To = Control; T1 = 1 h treatment of PDS; T2 = 2 h treatment of PDS; T3 = 3 h treatment of PDS; T4 = 4 h treatment of PDS.

noticed in root length (2.9%), RFW (0.03%) and in leaf area (72.1%) only (Table 1; Fig. 1). Plant derived smoke applied for 3 h also showed pronounced increase in root length (2.4%), shoot length (6.9%), RFW (0.10%), SFW (0.7%), SDW (0.13%) and in leaf area (81.9%) respectively. Plant derived smoke exposure applied for long time duration (4 h) had shown no significant results in all parameters studied.

The effect of plant derived smoke on physiological parameters had shown significant ($p \leq 0.05$) results. On comparison with control, plant derived smoke exposure for 1 h showed increase in RWC (11%), water potential (1.5%), osmotic potential (1.4%), MSI (4.3%) respectively (Table 2; Fig. 2). In 2 h PDS treatment significant increase was also noticed in RWC (17.3%), water potential (1.5%), osmotic potential (1.4%) and in MSI (4.3%) whereas 3 h and 4 h PDS treatment results increase in RWC (10.6%) (19%) and in MSI (14.6%) (12.3%) only. Results of the quantification of photosynthetic pigments indicated that all the smoke-related treatments (1 h, 2 h, 3 h and 4 h) significantly influenced the relative abundance of various photosynthetic pigments (Table 2; Fig. 2). Compared with the control, all the smoke-related treatments significantly ($p \leq 0.05$) increased the synthesis and/or accumulation of chlorophyll a relative to chlorophyll *b*, which in turn increased the chlorophyll a to chlorophyll *b* ratio significantly. The control plants had the lowest total chlorophyll and the chlorophyll a to b ratio whereas PDS treatment resulted in increase in chlorophyll a to b ratio and total chlorophyll contents. In comparison with the control, the chlorophyll a, b and total chlorophyll content concentration was increased with increasing PDS exposure and the maximum increase of 23.9% & 27.3% in chlorophyll a, 17.4% & 22.9% in chlorophyll *b* and 13.1% & 16.4% in total chlorophyll content was recorded in 3 h and 4 h PDS treatment (Table 2). Estimation of biochemical parameters per plant showed significant ($p \leq 0.05$) variation in response to the smoke treatments (Table 3; Fig. 3). In comparison with the control normal production of proline contents, free amino acids and soluble sugar was observed in all PDS treatments. A significant increase in proline contents (1.1%), free amino acids (0.4%) and in soluble sugars (1.7%) was recorded in 4 h PDS treatment only while in all other smoke treatments the production of these biochemical compounds was normal (Table 3).

4. Discussion

The use of sustainable agricultural practices and modern technologies involved in the preservation of resources are feasible option to increase agricultural productivity. The use of organic fertilizers, plant derived herbicides for seed or crop treatment are sensible example of efforts in the direction of sustainable agricultural practices. As a result there has been an increase in demand for such naturally derived agrochemicals for sustainable farming systems. In present study increase in

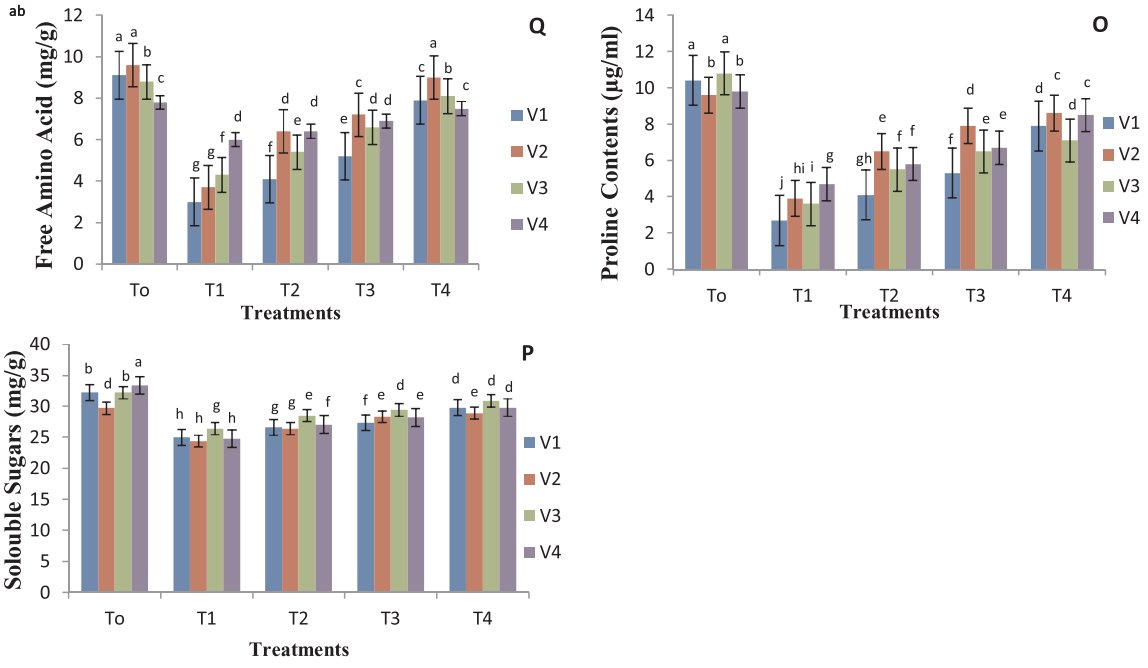

Fig. 3. (O,P,Q). Curves of selected physiological parameters showing various patterns in response to plant derived smoke treatments among wheat varieties. To = Control; T1 = 1 h treatment of PDS; T2 = 2 h treatment of PDS; T3 = 3 h treatment of PDS; T4 = 4 h treatment of PDS; V1 = NARC-11; V2 = Aas-11; V3 = Pak-13; V4 = Glaxy-13.

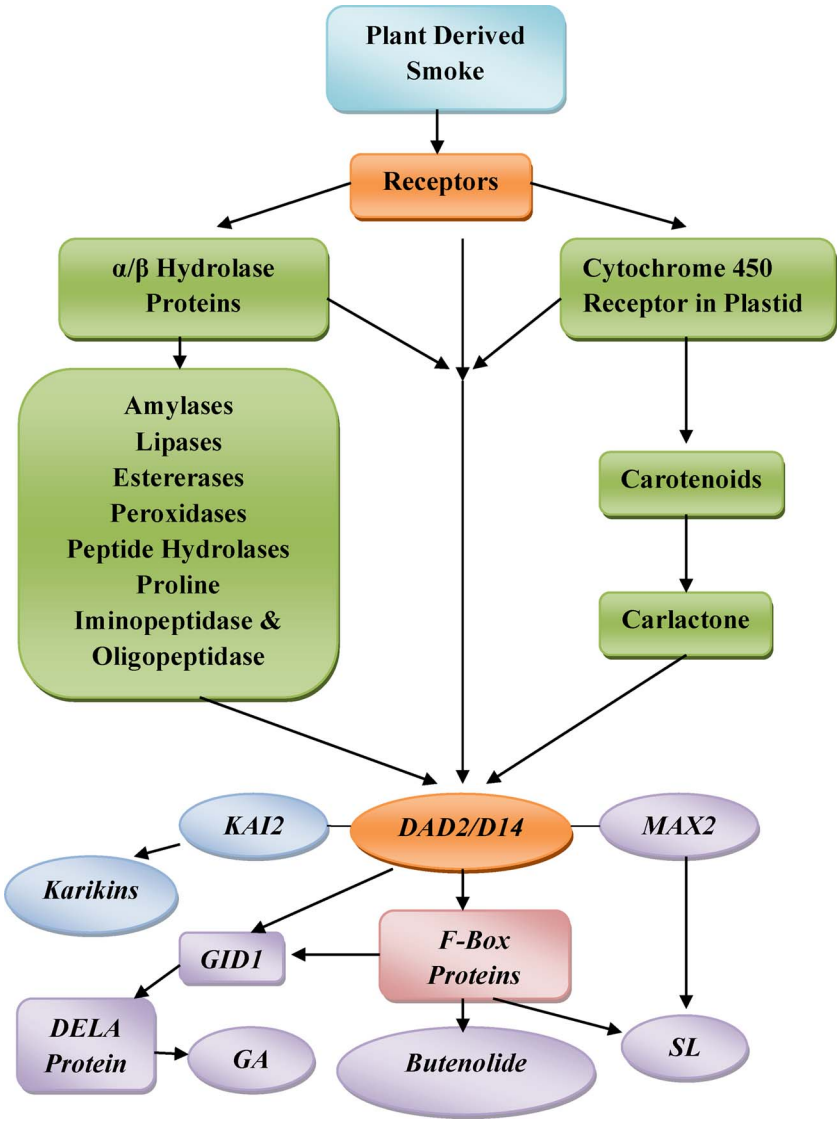

Fig. 4. Model illustrating the possible role of plant derived smoke as master regulator of various germination (Gibberellic Acid and Karikins) and growth stimulating hormones (Butenolides & Strigolactone) in wheat plants.

morphological attributes such as root shoot length, fresh and dry weight and leaf area was noticed in wheat plants. Increase might have been due to presence of butenolide having stimulatory effect on plant. Hence smoke produced from partial burning of plant material is effective in enhancing morphological growth. The positive impudence of smoke treatments on plant root and shoot was also reported by Zhou et al. [20] and Iqbal et al. [8]. This increase in root length is might be due to an increase in replicated DNA [21] initiation of the cell division cycle [22] and contribution of gibberellins [23]. Ghebrehiwot et al. [24] also reported increase in dry biomass of Tef grass in response to aerosol plant derived smoke treatments. A significant increase in leaf area was recorded in wheat plants in response to plant derived smoke. Aremu et al. [25] analyzed the positive effect of plant derived smoke on enhancement of leaf area in tissue cultured banana variety Williams.

Plant derived smoke treatments positively influenced physiological attributes of wheat plants such as relative water contents, water potential, osmotic adjustment, membrane stability index, chlorophyll a, b and total chlorophyll contents etc. Leaf relative water content is an important physiological parameter having close relationship with plant water potential [26]. In present research work amplification in leaf relative water contents was recorded in wheat plants in response to plant derived smoke (Table 2; Fig. 2). Habibi [27] reported increase in water contents in barley plants in response to SA treatment. Javid et al. [28] reported that growth hormones stimulate plants efficiency of water uptake, conservation and utilization. After leaf relative water contents, leaf water potential is considered as reliable parameter for computing plant water relationship. Plant derived smoke elicits stimulatory effect on water and osmotic potential and increase in both these parameters was observed in wheat plants in present study (Table 2; Fig. 2). Similarly Sayar et al. [29] reported increase in water and osmotic potential in wheat verities. Reason behind this increase is can be less uptake of Na^+, Cl^- and K^+ ions and reduction in deposition of organic osmolytes such as proline, free amino acids and soluble sugar contents respectively. Plant derived smoke act like the same way and butenolide present in smoke regulate these ions and production of osmolytes. Van Staden et al. [30] also explained that butenolide present in plant derived smoke positively affect physiological parameters by interacting with endogenous plant growth regulators. The stabilization and integrity of plant membranes is very important to maintain plant systems. Under normal conditions biological membranes retain their integrity and plant growth hormones play functional role in maintaining membrane stability. In this scenario plant derived smoke has also proved significant role due to presence of compound called butenolide which act like plant growth hormone. In present work increase in membrane stability index was observed in plants in response to plant derived smoke exposure. The reason behind this protective role is also described by Werner and Schmulling [31] who reported that plant hormones reduce production and stimulate degradation of free radicals

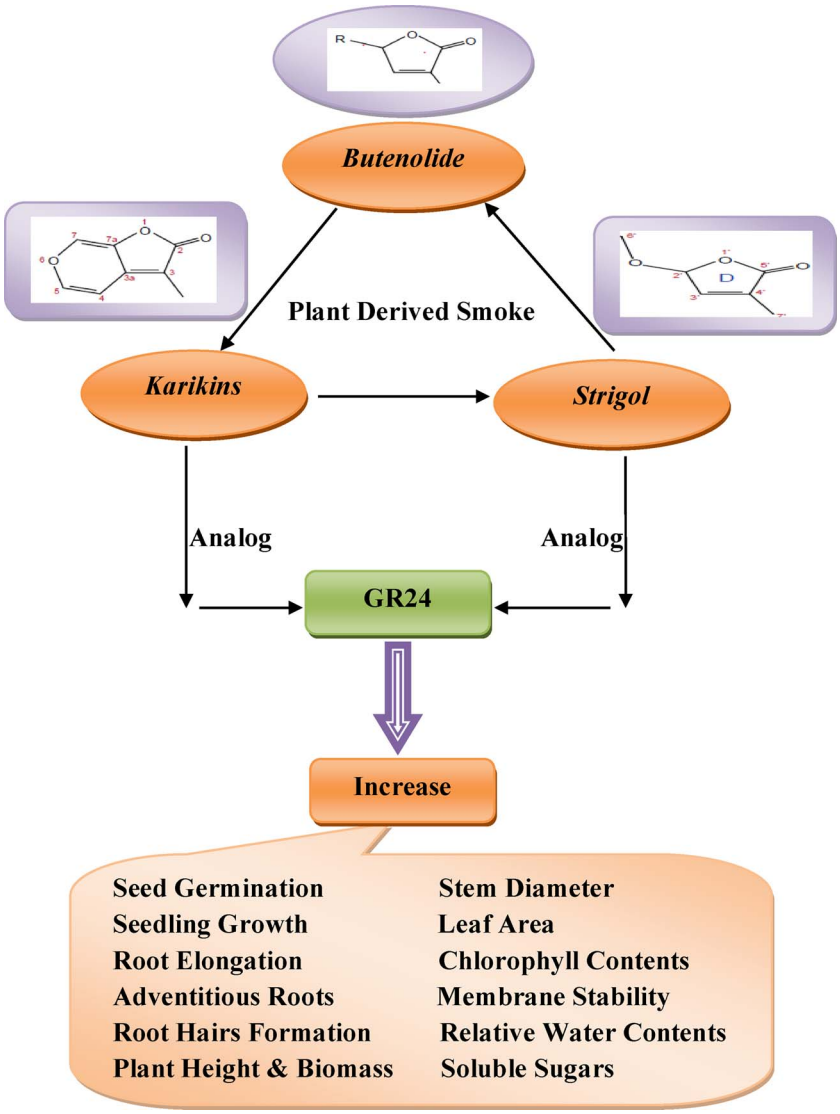

Fig. 5. The structures of biologically active compounds present in plant derived smoke and their possible role in growth and development of wheat plants.

such as superoxide (O) and hydroxy radical (OH) that cause damage to membrane lipids. Gupta et al. [32] also indicated promotery role of putrescine and BA treatment in increasing membrane stability of wheat.

The process of photosynthesis is essential for plant growth and development. It is affected by pigments such as chlorophyll which is an important catalyst of this process. Plant growth hormones are widely applied to enhance photosynthesis activity. Rao et al. [33] stated that increase in chlorophyll quantity contribute towards high production and improved yield of plants. The estimation of photosynthetic pigments and their consequent mutual percentage is a vital analytical tool that evaluates the greenness, senescence and on the whole plant growth environment. For example, chlorophyll a/b proportion is a sign of the functional pigment apparatus and light adjustment, whereas the total chlorophyll contents represent the greenness character in plants [34]. Aremu et al. [25] reported similar findings of increase in chlorophyll a/b contents in banana variety Williams, when subjected with plant derived smoke treatments. Similarly Ghebrehiwot et al. [35] also recorded profound increase in total chlorophyll contents in cereal crop *Eragrostis tef* after treatment with plant derived aerosol smoke. Xie et al. [36] and Gupta et al. [32] explained that rate of photosynthesis and rubisco activity was increased in wheat plants after treatment with BA and putrescine respectively.

In crops like wheat different type of compatible solutes (proline, free amino acids and soluble sugar contents) are produced and accumulated for osmotic adjustment, which is an adaptation to survive and grow better in different environmental conditions. Among these compatible solutes proline is one of major importance, play an effective role in protection of cell membrane and macromolecules structure in severe environmental conditions [37]. Proline helps to maintain the cell turgor potential [38], maintain protein structure, pH of the cell, may serve as metabolic or energetic reserve in plants [39,40] and also minimize the oxidative injury by scavenging reactive oxygen species [41]. In present research proline contents was decreased in response to plant derived smoke. Proline production in plants becomes less in response to plant growth hormones in normal conditions and it has been previously explained that plant derived smoke has similar activity like plant growth hormones. Results of present study revealed decrease in accumulation of amino acids in response to 1 h and 2 h plant derived smoke exposure but with increase of PDS exposure to 3 h and 4 h amino acid production becomes high. This increase in concentration of amino acids is due to hydrolysis of proteins, which is stimulated by change in osmotic adjustment, particularly the cleavage of structural protein into amino acids [42]. Our findings are also similar to Zhou et al. [20], who reported that plant derived smoke stimulates protein degradation and enhance secondary metabolites accumulation in plant species. Soluble sugars has essential role in plant metabolism which is used as a product of hydrolytic processes, as a substrate in biosynthesis processes, energy production and has also role in sugar sensing and signaling system

[43,44]. In present investigation soluble sugar contents was produced in less amount in short PDS exposure time where as in response to PDS exposure applied for longer time, a gradual increase in soluble sugar contents was recorded. Plants adopt different types of mechanism, one of which is the accumulation of different type of osmolytes such as soluble sugar [45].

Plant derived smoke is an astonishing cheap stimulating source for production of vital growth hormones like butenolide, karikins and strigolactones. These hormones are inter-convertable and promote various plant processes like seed germination and plant growth respectively. There are two major receptors which receive the signal from plant derived smoke to regulate synthesis of above mentioned growth hormones. One receptor is cytochrome 450 located in plastids and other is α/β hydrolase proteins which are of prime importance. In plastids the PDS stimulate the conversion of carotenoids into carlacton which ultimately trigger DAD2/D14, MAX2 and KAI2 genes. From other side various enzymes like amylases, lipases, peroxidases etc from α/β hydrolase proteins also stimulate these genes. Among these genes KAI2 and MAX2 genes are directly involve in synthesis of karikins and strigolactone but DAD2/D14 genes work in dual way. DAD2/D14 genes produce F-Box proteins acting as stimulant for strigolactone and butenolide synthesis and GID1 genes activation. GID1 genes produce DELA proteins which involve in gibberellic acid synthesis in wheat plants (Fig. 4). The produced hormones share common D-ring in their structure and are inter-convertable which vary plant to plant. It has been reported by Zwanenburg et al. [46] that there are some other compounds like GR24 which act as analog of these hormones. These hormones stimulate seed germination, seedling growth, root elongation [8], adventitious roots formation, synthesis of root hairs, increase plant height stem diameter and plant biomass respectively. Current investigation suggested that these hormones present in or produced by PDS exposure also enhance leaf area, chlorophyll contents, membrane stability, relative water contents and soluble sugars in wheat plants (Fig. 5).

5. Conclusion

It may be concluded that plant derived smoke exposure has positive and stimulatory effect on morphological, physiological and biochemical attributes of wheat plants. Plant derived smoke exposure applied for short time is more suitable and showed significant effect as compared to prolonged PDS exposure. Plant derived smoke can improve physiomorphic and biochemical outputs of a cereal crop, *Triticum aestivum* L. The short term (1 h and 2 h) cool aerosol-smoke pretreatment of wheat seeds before sowing produced healthier plants. Therefore, relatively simple and affordable techniques of aerosol smoke treatment of wheat seeds before sowing can be an option for the resource-limited wheat growers to improve their wheat production.

Conflict of interest

The authors declare no conflict of interests.

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