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IMPACT OF PGRS PRIMING ON GERMINATION AND SEEDLING PARAMETERS OF PEA AND PLANTAGO UNDER ARTIFICIAL MAGNETISM

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Abstract
Seed priming is widely recognized as a significant phenomenon for synchronizing germination and enhancing the vigor of seeds. Seeking the importance of priming, the influence of magnetic treatments in overall performance of plants cannot be undermined as well. In the present study, seeds of Pisum sativum L. and Plantago ovata Forssk. were primed with essential Plant Growth Regulators (PGRs) such as; Gibberellic acid (GA_3), Potassium nitrate (KNO_3), Salicylic acid (SA), Ascorbic acid (AsA), Hydrogen peroxide (H_2O_2) in combination with different artificial magnetic treatment (North, South and N/S) to document morpho-physiological attributes. A lab experiment was conducted under Complete Randomized Design (CRD) which consisted of 6 seed priming treatment along with control (no-priming) each with three replications. Seeds were primed with water (H_2O) and PGRs for 12 hours and the final harvest of seedlings was obtained for morpho-physiological analysis after 20 days of germination. Results portrayed statistically significant ($p < 0.05$) data by increasing the morphological characteristics (root/shoot length, number of leaves, root/shoot fresh weight and root/shoot dry weight). Similarly, photosynthetic pigments i.e., chl a and carotenoids recorded significant variations under artificial magnetism except chl b which showed non-significant ($p > 0.05$) variations. Results concluded that hydro priming and pre-soaking of seeds with essential PGRs are highly recommended to maximize the early germination, morpho-physiological performance in pea and plantago seedlings under artificial magnetism.
Keywords: *Artificial magnetism, Plantago, Isopghol, Secondary metabolites, Seed-priming*

INTRODUCTION

Plantago ovata Forssk. (Isopghol, jiru, and psyllium) is pharmaceutically important plant member of family Plantaginaceae which is herb that grow 10-40 feet in height (1). The number of leaves per plants varies 45-85, which are alternatively arrange, flowers are arrange on spikes with four spiral rows, 1 ovule and ovary is bilocular (2). It is grown regionally around the world due to it medicinally potential (3). Seeds of Plantago are major source of many secondary metabolites Such as Glycoside, sugars, plantiose and aucbin (4), while pea (*Pisum sativum* L.) is member of family Fabaceae (Legume) one of advance flowering plant family and cultivated worldwide, cold season annual herb crop. Legumes are the most important due to presence of protein and nutrient for both animals, human, enhance soil fertility by the symbiotic association with nitrogen fixing bacteria and boost the economy of the country (5).

In living environment change due to magnetic field is crucial and many changes are induced in growth and development of living organisms; to investigate the impact of magnetic field on living body, plants are best tool (6). The prevailed literature is available on electromagnetic treatment on plants with profound results under environmental stress condition; temperature, salinity, and drought (7, 8). Germination of seeds stimulated when treated with optimal electromagnetic field. Magnetic field enhances the seed potential energy that directly responses in stimulating metabolism which improve germination mechanisms of seed (9). Seed vigor, germination, seedling growth improved, and protein contents and antioxidant defense mechanism stimulated by electric and magnetic field treatment (10-12). The germination, growth and yield parameter of Mung beans variety improved under electric field pre-treatment positively (13). Plants such as cucumber, maize, lettuce, radish, and tomato seeds were exposed/ treated with electric filed, showed increase in germination rate of seeds and seeds vigor index (14-17). Chickpea seeds exposed to MF, show improved seedling growth performance, germination rate and dry biomass of seedling with respect to duration and strength of MF (18).

Seed priming is one new approach in improving the seed germination and growth of crop plants (19). It activates metabolic process in seed that result in stimulation of seed germination and seedling growth at early stage (20). Plant growth regulators are molecules that positively regulate growth and development, from seed germination to plant fruiting stage, in various species of plants. In many crops seed dormancy stimulated by application of gibberellin (21). These are chemical messenger produced in small concentration and control many morphological, physiological, biochemical, anatomical, and molecular mechanism in plants (22, 23). In plant various morpho-physiological, and biochemical process are control by gibberellin such as, cell division, plants growth in height, elongation, rate of transpiration, flowering, and leaf expansion (24). Samad *et al.,* (25) reported that PGRs application enhances the plant photosynthetic pigments efficacy, secondary metabolites contents and decreases hydrogen peroxide and MDA under salinity stress.

Salicylic acid is plant growth regulator that consist of diverse phenolic compounds and aromatic ring synthesized in plants. SA induces tolerance when plants exposed under abiotic stresses (26, 27). Foliar application of SA enhances enzymatic antioxidants activates (CAT, SOD and POD), soluble sugar contents, Glycine betaine, proline and root and leaf K^+ while decrease the malondialdehyde, hydrogen peroxide and Na^+ (28). Similarly, hydrogen peroxide (H_2O_2) is one recognized chemical, has ability to induce tolerance under abiotic and biotic environmental condition, being a signaling molecule play vital role in plants acts a messenger in signaling pathways, trigger stress tolerance in plants against environmental stress (29). Seed priming and foliar application of SA and Hydrogen peroxide under Cd^{+2} Stress improved plants morpho-physiological, biochemical parameters at two different altitudes grown milk thistle (27).

Vitamin C is one known as an antioxidant, a cofactor of enzyme and tartrate and oxalate precursor. Ascorbic acid is chloroplast affiliated vitamin which mitigate oxidative stress that are related to the photosynthetic apparatus, it also detoxifies hydrogen peroxide and abates the alteration in cell division as primary substrate. Vitamins are known as hormones precursors or bioregulators compounds in small concentration shows a valuable impact on plants development and overall, these affect the plant metabolic energy pathways (30, 31). Various plants essential physio-chemical processes such as biosynthesis of enzymes, plant photosynthesis, water and nutrient absorption, secondary metabolites production and cell division depend on availability of vitamin, vitamins also activate plants defense under oxidative stress (32).

In addition to the traditional tool's, foliar application of different organic substance and minerals contributed to mitigate abiotic stress as water deficiency in plants (33, 34). Supplementation of nitrogen compounds (KNO_3) improves the activities of enzymatic antioxidants and cell osmotic adjustment, photosynthesis, stomatal conductance, and plants nutrition under stress (35). The purpose of the present experiment was to evaluate the priming potential of different plant growth regulators in enhancing germination of seeds, growth and photosynthetic pigments of *Pisum sativum* and *Plantago ovata* seedlings in response to artificial magnetism.

METHODOLOGY

EXPERIMENTAL DESIGN

A lab experiment was conducted to assess the impact of seed priming in response to magnetic fields. Plantago and Pea plants seeds were primed with H_2O, Hydrogen peroxide (H_2O_2), Salicylic Acid (SA), Ascorbic Acid (AsA), Gibberellic Acid (GA_3), Potassium Nitrate (KNO_3) for 12 hours. After priming, seeds were placed in petri dishes labelled w.r.t various magnetic treatments. Furthermore, different treatments of artificial magnetism as described by (36) with certain modification was used in comparison to control in

Petri dishes. Screening of each compound/hormone was done prior to final setup of the experiment to select the best one level. After 20 days of germination, plants were harvested and data was collected to record various Morpho-physiological attributes for both plants. The design for this research experiment was Completely Randomized Design (CRD) with three replications.

SOURCES OF SEEDS

The certified and viable seeds of Pea and Plantago were provided by Balochistan Agricultural Research and Development Centre (BARDC), Quetta.

DETERMINATION OF GROWTH ATTRIBUTES

For the determination of morphological parameters, root and shoot length was observed in centimeter (cm) by using a scale. The total number of leaves were counted w.r.t various treatment. Shoot and root fresh weight was noted using an electric balance right after harvest. While shoot and root dry weights were noted after the samples were oven-dried at 65-75°C for 24 hours.

PHOTOSYNTHETIC PIGMENTS ANALYSIS

Leaf samples (0.1 g) were extracted with acetone (80%). The absorbance as recoded at 663 nm, 645 nm and 480 nm against 80% acetone (37, 38). Chlorophyll *a*, *b* and carotenoids contents were measured using the following formulas:

Chlorophyll *a* (mg/g fresh wt.) = (1.27 (OD663)-2.69 (OD645) × V / 1000×W

Chlorophyll *b* (mg /g fresh wt.) = (22.9 (OD645)- 4.68 (OD663) × V/1000×W

Carotenoids (mg/g fresh wt.) = (OD480 + 0.114 (OD663) - 0.638 (OD645) / 2500 × 1000

STATISTICAL ANALYSIS

The recorded data was statistically analyzed using “STATISTIX 8.1”. (Analytical software, Tallahassee, Florida), and the mean, SD, and graphs were generated by using the MS EXCEL. Furthermore, a post hoc test such as the least significant difference (LSD) was used, and the labels/alphabets were added to the graph bars of statistically significant attributes ($p < 0.05$).

RESULTS

MORPHOLOGICAL PARAMETERS

ROOT LENGTH

Data recorded for root length of two plant seedlings *i.e.*, pea and plantago reported statistically ($p < 0.05$) significant results. Data further revealed that priming of pea plants with GA_3 + south magnetism treatment (12.50 cm) showed highest root length, while in control (no-priming) + north magnetism (2.50 cm) showed least root length. Similarly, in plantago, control + south magnetism (2.15 cm) treatment was the best in having highest root length, while priming of AsA + N/S magnetism (01.30 cm) treatment showed least root length (Fig. 1A).

In a nutshell, it has been noted from the root length parameter that GA_3 primed seed showed more significant results increasing the root length in pea plants as compared to plantago which exhibited high root length in AsA primed seeds under N/S magnetic treatment (Fig. 1A).

SHOOT LENGTH

Results obtained for shoot length of pea and plantago seedlings, illustrated highly significant data statistically ($p < 0.05$) (Fig. 1B). Maximum shoot length was observed in H_2O_2 priming + north magnetism (24.00 cm) in pea seedlings, and the minimum shoot length was observed in control + north magnetism (9.50 cm). However, in comparison it has been observed in plantago seedlings that maximum shoot length was recorded in H_2O_2 priming + north magnetism (04.50 cm) while the minimum value for shoot length was observed in GA_3 priming + N/S magnetism treatment (02.50 cm).

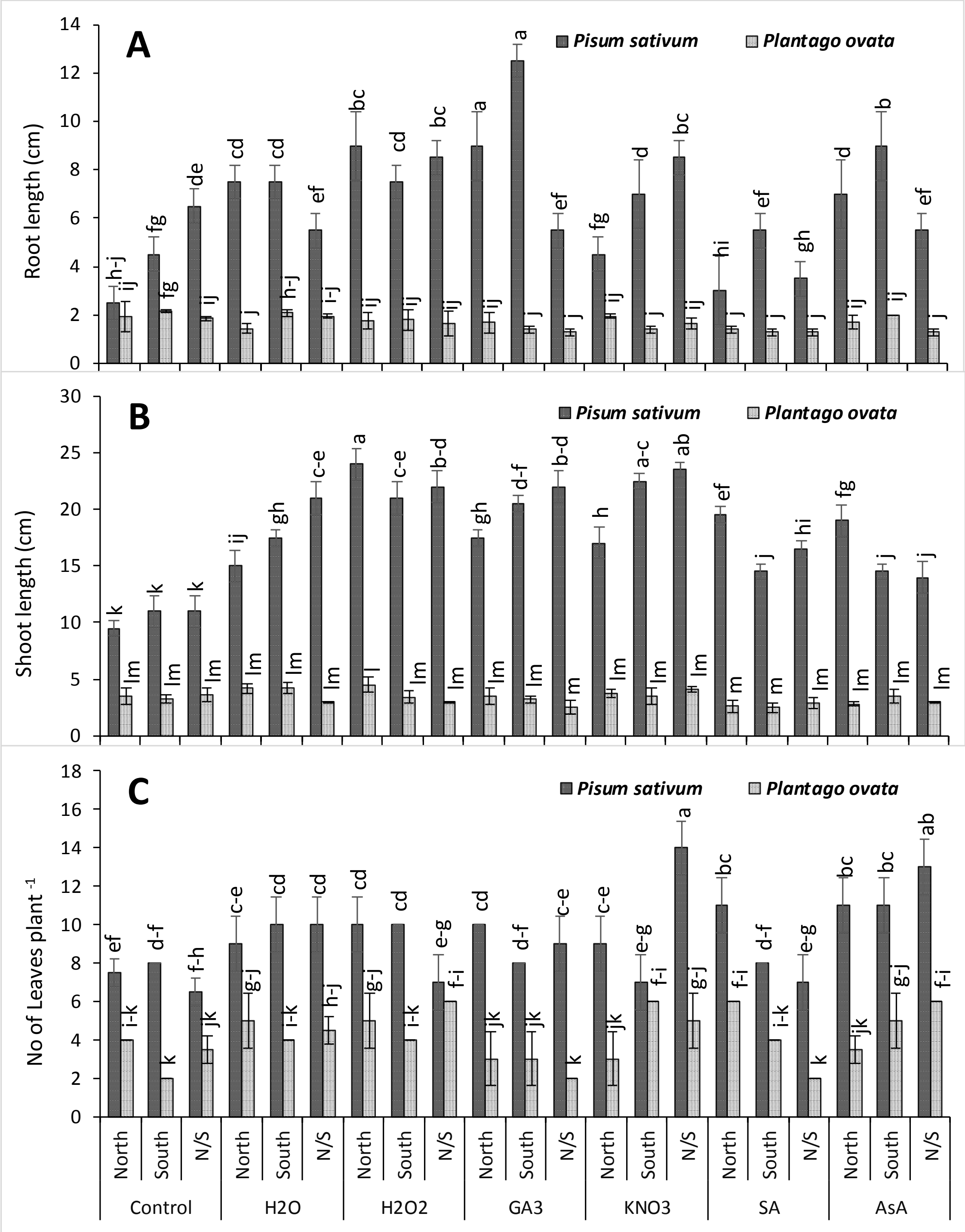

Fig. 1. Effect of different magnetic treatments on Growth attributes; Root length **(A)**, Shoot length **(B)** and Number of leaves^{-1} **(C)** of *Pisum sativum* L. and *Plantago ovata* in response to priming of various PGRs. Different alphabets/letters on bars represents statistically significant interactions ($p < 0.05$) according to the least significant difference test.

From the overall results, it was observed that priming of pea and plantago seeds with H_2O_2 improved the shoot length under north magnetic treatment as compared to other treatments followed by priming of seeds with KNO_3, SA, AsA, H_2O_2 etc. Therefore, seed priming with essential growth chemicals will boost up the germination process along with enhancement of morphological parameters attributes (Fig. 1B).

NUMBER OF LEAVES PLANT^{-1}

Data for number of leaves in plantago and pea seedlings, also showed statistically significant ($p < 0.05$) results. For pea, the data revealed that maximum number of leaves was counted in KNO_3 priming + N/S magnetism (14) while minimum leaves number was noted in control + N/S magnetism (6.50) treatment; likewise, in plantago, it was observed that highest leaves were counted in SA priming + north magnetism (6) while minimum number of leaves was counted in SA priming + N/S magnetism (2) treatment (Fig. 1C).

Results revealed that seed-priming with PGRs help seedlings to germinate earlier and produce more leaves in comparison with non-treated seeds (no priming) (Fig. 1C).

ROOT FRESH WEIGHT (RFW)

The results for RFW in pea and plantago revealed statistically significant ($p < 0.05$) data by describing that in pea seedlings, the best treatment was recognized as H_2O priming + N/S magnetism (0.17 g) treatment and the least RFW was observed in SA priming + north magnetism (0.10 g). Furthermore, in plantago seedlings, priming of plants with concentration of AsA + south magnetism (0.18 g) highly improved the fresh weight of root while in the same plant (Plantago), the least RFW was observed in H_2O priming + N/S magnetism (0.08 g) (Fig. 2A).

Concludingly, considering the RFW, it can be said that AsA is one of the most essential PGRs which in combination with south magnetism improved the fresh weight of root (Fig. 2A).

SHOOT FRESH WEGIHT (SFW)

Results obtained for SFW of pea and plantago, showed statistically significant ($p < 0.05$) results. Data further highlighted that in pea plants, priming of seeds with H_2O_2 + N/S magnetism (0.32 g) improved the fresh weight of shoot as compared to other treatments and the lowest SFW was observed in AsA priming under N/S magnetism (0.15 g). Moreover, in plantago maximum SFW was recorded in AsA priming + south magnetism (0.21 g) treatment, while minimum SFW was observed in control conditions under the north magnetism (0.11 g) treatment (Fig. 2B).

Overall results for SFW revealed that greater fresh weight for shoot of pea plants was due to the priming of seeds with H_2O_2 under N/S magnetic treatment as compared to plantago. While in plantago, control conditions showed significant but low weight of shoot under north magnetism treatment (Fig. 2B).

ROOT DRY WEGIHT (RDW)

Data recorded for RDW in pea and plantago seedlings illustrated statistically significant ($p < 0.05$) variations in overall PGRs treatment under magnetic treatments. In pea seedlings, the maximum RDW was observed in KNO_3 priming + south magnetism (0.06 g) and the minimum weight for dry root was recorded in control under south magnetism (0.02 g) treatment. Results for plantago seedlings further revealed that maximum dry weight in root was observed when seeds were primed with H_2O priming + south magnetism (0.05 g), while a low level of root dry weight was recorded in the same plant (plantago) in AsA priming + N/S magnetism treatment (0.02 g) (Fig. 2C).

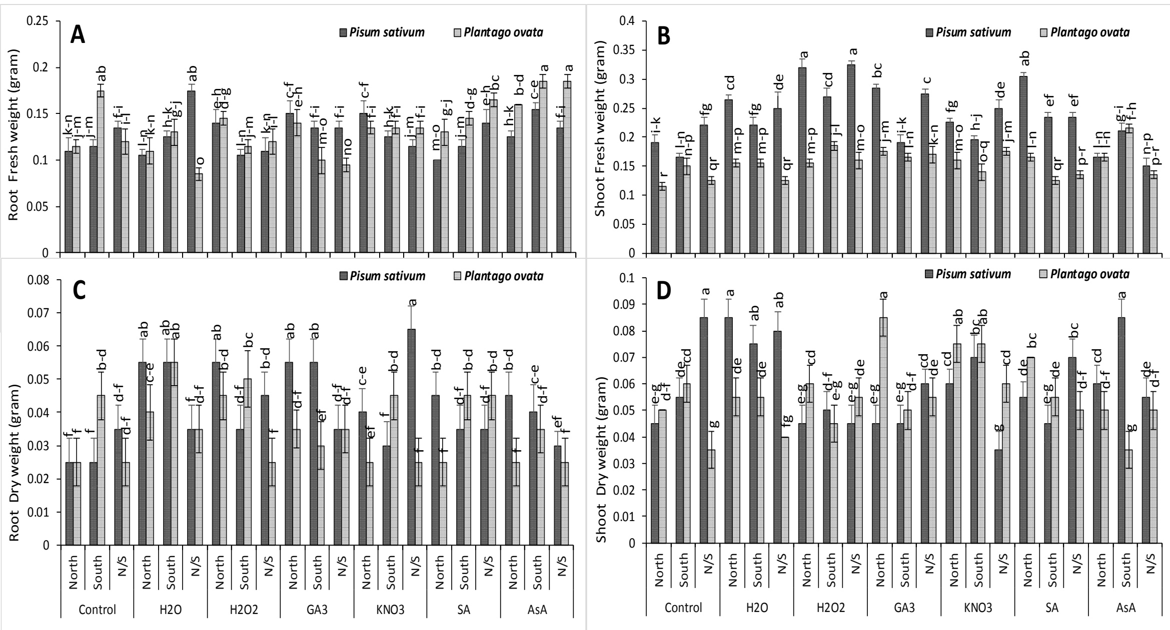

Fig. 2. Effect of different magnetic treatments on Growth Attributes; Root fresh weight **(A)**, Shoot fresh weight **(B)**, Root dry weight **(C)**, Shoot dry weight **(D)** of *Pisum sativum* L. and *Plantago ovata* in response to priming of various PGRs. Different alphabets/letters on bars represents statistically significant interactions ($p < 0.05$) according to the least significant difference test.

Summing up the overall results, it's astonishing to note that in plantago, priming of seeds with KNO_3 highly improved the dry weight of root, which illustrate that plantago is capable of germinating so fast rate by developing essential root system under N/S treatment as compared to pea plants (Fig. 2C).

SHOOT DRY WEIGHT (SDW)

SDW in response to different priming treatments under magnetism treatments also described significant ($p < 0.05$) variations in pea and plantago. Data further revealed maximum dry weight of shoot in pea plants by the priming of H_2O + north magnetism (0.08 g) treatment, while minimum SDW was recorded in KNO_3 priming + N/S magnetic treatment (0.03 g). Moreover, GA_3 priming + north magnetism (0.08 g) treatment in plantago recorded the highest SDW, while control + N/S magnetic (0.03 g) treatment showed the lowest SDW (Fig. 2D).

In a nutshell, it has been recognized that north magnetism treatment was effective in accumulating dry weight of shoot under water primed seeds of pea plants. This shows that water primed improved the SDW as compared to other PGRs treatment. While in plantago, the primed application of KNO_3 with seeds got much effected in enhancing the SDW (Fig. 2D).

DETERMIANTION OF PHOTOSYNTHETIC PIGMENTS

CHLOROPHYLL A (Chl a)

Data recorded for chl *a* in both plantago and pea plants showed statistically significant ($p < 0.05$) variations. Data revealed in plantago seedlings, priming of seeds with SA under south magnetism (4.66 mg/g FWT) treatment showed maximum chl *a* content and the minimum amount of chl *a* was recorded in KNO_3 priming under N/S magnetism (1.26 mg/g FWT). Hence, considering the pea seedlings, it was observed that SA priming + south magnetism (4.34 mg/g FWT) showed highest chl *a* content while the lowest content was noted in H_2O_2 priming + north magnetism (0.56 mg/g FWT) treatment (Fig. 3A).

Considering the overall improvements in plantago and pea seedlings, data suggest that plantago accumulated highest chl *a* content as compared to pea seedlings (Fig 3A).

CHLOROPHYLL B (Chl b)

Results recorded for chl *b* of pea and plantago seedlings statistically revealed non-significant ($p > 0.05$) variations. The data further illustrated that H_2O priming of plantago seeds under north magnetism treatment (3.01 mg/g FWT) showed maximum chl *b* content while H_2O_2 priming + N/S magnetism (0.66 mg/g FWT) treatment showed the least value in obtaining chl *b* content. Moreover, in pea seedlings, the maximum chl *b* content was observed in control + N/S magnetic treatment (2.54 mg/g FWT), while GA_3 priming + North magnetism (0.26 mg/g FWT) showed minimum chl *b* content. Which suggest that both plants showed remarkable variations in the accumulation of photosynthetic pigments (Fig. 3B).

Application of GA_3 and H_2O_2 was considered to be very effective in the highest accumulation of chl *b* content in plantago seedlings as compared to other PGRs. Therefore, the overall trend suggests that priming of pea and plantago seedlings with various growth regulators highly improve the chl *b* content (Fig. 3B).

CAROTENOIDS

Data obtained for carotenoids of plantago and pea seedlings showed statistically significant ($p < 0.05$) variations. The data further revealed that highest carotenoids content in plantago seedlings was accumulated in GA_3 primed treatment under north magnetism (0.91 mg/g FWT), while lowest carotenoids content was observed in control conditions (without priming) under south magnetism (0.23 mg/g FWT). However, in pea seedlings, AsA priming + North magnetic treatment (0.72 mg/g FWT) showed highest carotenoids content and the least amount of carotenoids content was recorded in GA_3 priming + South magnetism treatment (0.18 mg/g FWT) (Fig. 3C).

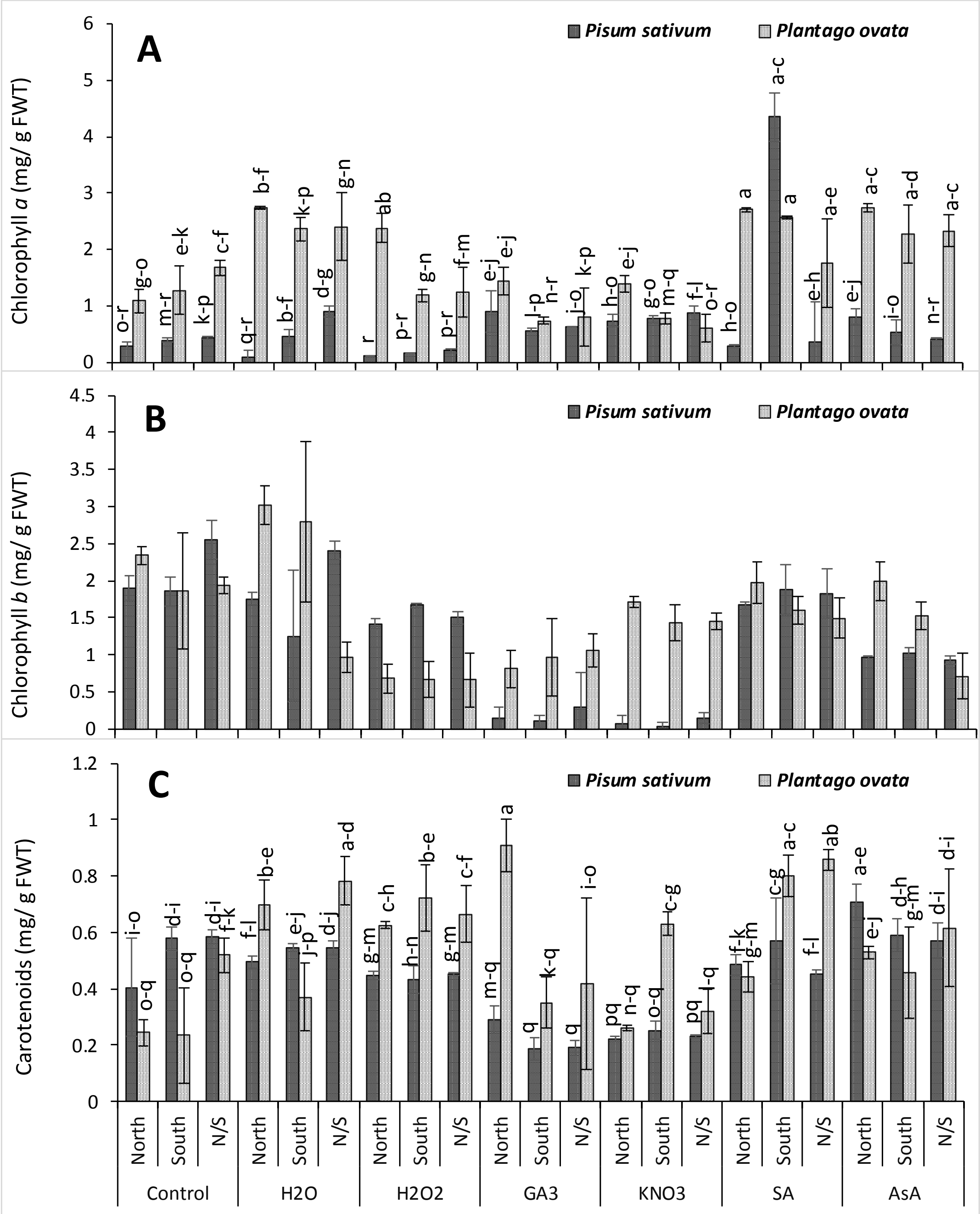

Fig. 3. Effect of different magnetic treatments on the photosynthetic pigments; Chlorophyll *a* **(A)**, Chlorophyll *b* **(B)** and Carotenoids **(C)** of *Pisum sativum* L. and *Plantago ovata* in response to priming of various PGRs. Different alphabets/letters on bars represents statistically significant interactions ($p < 0.05$) according to the least significant difference test. Bars lacking the labels show non-significant interactions at ($p > 0.05$).

Finally, the synergistic effects of PGRs specially GA_3 and AsA were very helpful in the accumulation of maximum carotenoids content in both plants. Overall photosynthetic parameters portrayed the essential role of seed-priming with PGRs can be highly influential in the morpho-physiological variations to a great extent (Fig. 3C).

DISCUSSION

The purpose of the present study was to evaluate the potential and valuable role of plant growth regulators in combination with different magnetic treatments to promote the early germination, seedlings growth and morphophysiological attributes of pea and Plantago seedlings. Diverse studies by utilizing the magnetic applications treatments have shown that magnetism has positive potential in the morphological parameters of plants *i.e.*, (root/shoot length, number of leaves, root/shoot FW, root/shoot DW) (39, 40). The treatment of magnetic field, root and shoot weight highly enhanced in Lupin (41). Similarly, the present study also used the magnetic application (North, South and N/S) in combination with priming of seeds with different PGRs for the better vegetative growth of pea and plantago plants. It has been observed that in pea, GA_3 under south magnetism showed highest root length, H_2O_2 priming under north magnetism in pea showed highest shoot length, SA under N/S magnetic treatment improved the total number of leaves (Fig. 1A-C), H_2O_2 under N/S magnetic treatment in pea enhanced the SFW, AsA under south magnetism in plantago increased the RFW, KNO_3 under N/S magnetic treatment improved the RDW as compared to control conditions (Untreated seeds with PGRs and magnetism) (Fig. 2A-D). Morphological attributes are the basic parameters of plants that play significant role in overall functioning of plant growth and development throughout its life cycle. Initial stages of the development and growth of plants especially pea plants are more susceptible to salinity and other type of stresses (42).

Keeping in view the overall functioning and growth of plants organs, plant growth regulators (PGRs) are considered to be very much efficient in enhancing the both seed vigor and growth of seedlings under stressful environment. Naseer *et al.*, (43) reported that the application of artificial magnetism treatment, when applied to pea plants, significantly enhanced the shoot length (35.29%) and root length (63.41%) over geomagnetism treated pea plants. Similar findings were also reported by Nizar *et al.*, (27) that priming and foliar supplementation of H_2O_2 enhanced the germination process along with the astonishing increase in morphological characteristics of milk thistle under cadmium stress. Indeed, in the present experiment, similar findings portrayed the essential role of south and north magnetism with the collaboration of GA_3 and H_2O_2 highly improved the length of root and shoot in both plants (Fig. 1A and B).

Hussain *et al.*, (44) reported that vegetative growth attributes *i.e.*, shoot and root length, root and shoot fresh and dry weight in two varieties (Meteors and Classic) of pea plants showed an astonishing and exclusive growth in plants. The seeds of these two varieties were primed with 0.1 mM and 0.2 mM AsA. The research experiment of Afzal *et al.*, (45) also reported the same that AsA supplementation mitigated the impacts of abiotic stress in plants. Singh & Dhingra, (46) reported that metabolic activities in the process of germination boosted up much earlier in soaked seeds than the radicle emergence. In different presoaking of seeds with H_2O (hydro priming) in American cotton helped to maximize the morphological attributes by increasing in their length, weight and size etc. Basra et al., (47) reported that hydro priming of wheat and sunflower seeds enhanced not only the germination process but also produced longer seedlings under NaCl stress as compared to those seeds which were not primed with H_2O. In the current experiment, results also support that in different morphological parameters hydro priming proved to enhance the morpho-physiological characteristics such as., RFW and SDW in pea seedlings, RDW in plantago, Chl *a* and soluble phenolics in plantago and soluble sugar in pea seedlings (Fig. 2A, C and D). Likewise, the experiment of Shatpathy et al., (48) suggested that a severe reduction was observed in the germination of seedling growth of rice (*Oryza sativa* L.) when the stress level is increased, but there are ways in which PGRs can improve the germination process. Similarly, priming of seed with salicylic acid (SA) of 100 ppm enhanced dry weight of seedling as compared to the mean germination of seeds which were untreated with the SA. Therefore, the SA-primed seeds of rice significantly increased shoot and root length in comparison with non-primed seeds. Exogenous supplementation of Fe is considered to be very important in the increasing of number of leaves in various plant species (49). However, the present findings suggest that priming of KNO_3 under N/S magnetic treatment showed greatest number of leaves in pea plants as compared to other PGRs along with the plantago which showed greatest number of leaves in SA priming under the same magnetic treatment (N/S) (Fig. 1C).

Chlorophyll is one of the most essential components in relation to photosynthesis. A significant reduction in Chl *a* and Chl *b* content of wheat cultivars was found (50) which cause metabolic imbalance and increase the degradation process of chlorophyll under drought stress (51, 52). Moreover, in *Pisum sativum* L. seedlings, a significant decrease in chlorophyll content was reported by the drought stress (53). Research experiment conducted by Naseer *et al.*, (43) suggested that under control conditions, Chl *a* remained high under artificial magnetic treatments in pea plants but foliar application of SA enhanced the content of chlorophyll *b* in response to different treatment of artificial magnetism, which was also supported by Karataş *et al.*, (54) that chlorophyll enhanced under magnetic field in maize and potato. Application of PGRs very profound in enhancing the overall photosynthetic parameters (Chl *a*, *b* and carotenoids) under salinity stress in plants. While Zahra *et al.*, (55) study suggested that Chl *a*, *b* and carotenoids contents increased by the foliar supplementation of PGRs under NaCl stress. The present findings are statistically similar with the above findings, but in the current study, the vital use of PGRs in priming of plantago and pea seeds for 12 hours was observed rather than foliar supplementation. Thus, PGRs have a potential role in early germination as well as play a crucial role in increasing the total amount of photosynthetic pigments such as; chl *a*, *b* and carotenoids as compared to non-treated seeds under control conditions (Fig. 3A-C).

CONCLUSION

The main focus of the current study centers around the potential benefits of priming of seeds with different PGRs under magnetic treatment (South, North and N/S) on morpho-physiological characteristics. It was observed that vegetative parameters such as; root and shoot length, root and shoot fresh and dry weight were significantly increased under PGRs treatment (Priming). Similarly, enhancement in physiological characteristics like; chl *a*, *b* and carotenoids was recorded in response to the seed-priming with various PGRs under magnetism treatment as compared to control. The present study, therefore highly recommend the utilization of growth supporting chemicals applications like (GA_3, KNO_3, AsA, SA, H_2O_2) through various routes in shape of priming would astonishingly help plants especially pea and plantago to enhance growth attributes. The seed-priming will not only initiate the germination process but more importantly mitigate the negative impacts of drought, mineral deficiencies in soil, salinity, water logging, global warming, heavy metal stress.

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