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GERMINATION AND GROWTH ANALYSIS OF WHEAT, BARLEY, AND OAT FOR HEALTH BENEFIT COMPONENTS IN GRAIN

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Abstract: Cereal grains provide half of the calories consumed by humans. In addition, they contain important compounds beneficial for health. During the last years, a broad spectrum of new cereal grain-derived products for dietary purposes emerged on the global food market. Special breeding programs aimed at cultivars utilizable for these new products have been launched for both the main sources of staple foods (such as rice, wheat, and maize) and other cereal crops (oat, barley, sorghum, millet, etc.). The breeding paradigm has been switched from traditional grain quality indicators (for example, high bread making quality and protein content for common wheat or content of protein, lysine, and starch for barley and oat) to more specialized ones (high content of bioactive compounds, vitamins, dietary fibers, and oils, etc.).

Keywords: *barley; breeding; marker-assisted selection; genes; genetic resources; genome editing; health benefits; metabolomics; oat; QTL; wheat*

Introduction

Cereal crops are the main food and feed sources worldwide, supplying more than half of the calories consumed by humans. An overwhelming majority of plant breeders and geneticists work on no other crops but cereals. Breeding methods depend on the biological features of a crop and on the genetic research standards, traditions, economic objectives, and levels of agricultural technologies in the country where plant breeding is underway. The general breeding trend of the past decades, however, was finding solutions to the problem of higher yields in cereal crops; furthermore, special attention was paid in many countries to increasing plant resistance against diseases and various abiotic stressors. The concentration of all efforts on these two targets and none other resulted in a certain decline in the genetic diversity in those plant characters that are associated with the biochemical composition of cereal grain.

The true grasses are monocot (Class Liliopsida) plants of the Family Poaceae (formerly Gramineae). There are some 600 genera and perhaps 10,000 species of grasses. This family includes a great many species of considerable ecological and economic importance, such as lawn and forage grasses, wheat, rice, maize, corn and oat. Agricultural grasses grown for food production are called *cereals*.

Cereals constitute the major source of calories for humans as rice in India and the Far East, maize in Mexico and wheat and barley in Europe and North America. Staple food grains are often called corn.

Materials AND Methods

Present investigation was undertake to analyse some biochemicals in five different selected cereals seedlings of the family Poaceae under different ages. Their seeds were obtained from local supplier - National Seed Corporation (NSC).

Selection of plants

Considering the literature, five members of family Poaceae were selected for the present study.

1. Wheat – *Triticum aestivum* L.
2. Corn – *Zea mays* L.
3. Barley – *Hordeum vulgare* L.
4. Oat – *Avena sativa* L.
5. Rice – *Oryza sativa* L.

Experimental Studies

Germination analysis

Healthy, uniform and viable seeds/fruits of different five crop wheat, rice, maize, oat and barley were surface sterilized. For this they were treated with 0.1% mercuric chloride for 30 seconds and willhed with sterile double distilled water 4-5 times for 10-15 min. Surface sterilized seeds were then allowed to imbibe distilled water for 6 hours and thereafter sown on Petri plates lined with cotton over which Whatman no. 40 filter paper was placed. Seeds were placed equidistantly on filter paper. For each variety five sets each having 100 seeds were maintained. Seeds were allowed to germinate. One to two mm length of radicle was considered as a sign of germination. Counting of germinated seeds was done daily until there was no further increase of germinated seeds. Germination behaviour was observed after 3, 5, 7, 10 and 15 days respectively.

Growth analysis

Seedlings of test plants were taken out from the Petri dishes and placed in wet paper towels and weigh for their length. Length of seedlings was also recorded. For dry weight estimation first excess water was removed from plant by using absorbent paper towel and seedlings of different crops were placed in a labelled paper envelope. They were placed in an oven at 80^0C for 24 h. Thereafter seedlings were weighed for their dry weight.

Moisture content (%)

Following formula was used to determine moisture percent content in root

$$= \frac{\text{FW of root} - \text{DW of root}}{\text{FW of root}} \times 100$$

Biochemical analysis

Following biochemical components were extracted from the seedlings of the test crops at their age of 3rd, 5th 7th, 10thand 15th days.

Leaf extract pH

Leaf extract pH was calculated with the help of digital electronic pH meter by homogenizing 5gm fresh leaves with 25 ml double distilled water.

Chlorophyll

Chlorophyll content was estimated according to Arnon's (1949) method. According to this method, fresh leaves (100 mg) were homogenised with chilled acetone (80%) and a pinch of sodium biocarbonate. The homogenate was centrifuged for 5 minutes and final volume of supernatant was made to 10 ml by adding 80% Acetone. The optical density (OD) of the extract was recorded at 645 and 663 nm on a spectrophotometer against a blank (80% acetone). The amount of chlorophyll *a*, chlorophyll *b*, and total chlorophyll was calculated using the following formulae :

$$\text{Chlorophyll } a = (12.7\ A_{663} - 2.69\ A_{645}) \times \frac{V}{W \times 1000}\ (\text{mg g}^{-1}\ \text{f. weight})$$

$$\text{Chlorophyll } b = (22.9\ A_{645} - 4.68\ A_{663}) \times \frac{V}{W \times 1000}\ (\text{mg g}^{-1}\ \text{f. weight})$$

$$\text{Total Chlorophyll} = (8.02\ A_{663} - 20.2\ A_{645}) \times \frac{V}{W \times 1000}\ (\text{mg g}^{-1}\ \text{f. weight})$$

Where V = Final volume of sample (ml)

W = Fresh weight of sample (g)

A = Absorption

Fractionation of chlorophyll:

Thin layer chromatography (TLC) in different solvent systems was tried for fractionation of chlorophyll. Thin layer chromatography was found more appropriate than the paper-chromatography. So fractionation of chlorophyll was done by thin layer chromatography. TLC slides are prepared from Silica-gel-G, by mixing 20 ml of distilled water and 50 gm of silica gel.

Slides were activated at 120^0C. Before their use, 0.5 ml of the ether extract was applied on a slide and after drying, slide or chromatogram development, number of colour, and Rf values of different bands were recorded. Chromatograms were developed in dark at room temperature (35-37^0C). The best solvent for fractionation of chlorophyll by thin layer chromatography was found to be Benzene: Acetone 8.5: 1.5. Total five bands were obtained within 20-25 minutes, which are designated as chlorophylls.

Carotenoids

Carotenoids were estimated according to method the devised by Maclachlan and Zalic (1963). For this 100 mg fresh tissue was homogenised in chilled acetone (80%) solution and centrifuged at 6000 rpm. After centrifugation, supernatant was taken and final volume of supernatant was made upto 10 ml with the help of 80% acetone. Samples were kept in cool and dark place for overnight. Next day optical density was measured at 480 nm and 510 nm with the help of spectrophotometer. Amount of carotenoids was calculated with the help of following formula-

$$\text{Carotenoids} = (7.6\ A_{480} - 1.96\ A_{510}) \times \frac{V}{W \times 1000}\ (\text{mg g}^{-1}\ \text{fresh weight})$$

Where V = Volume of sample (ml)

W = Weight of fresh tissue (g)

A = Absorption

RESULT

The Objective of the present studies was to extract some biochemical components in the seedlings of wheat, maize, oat, rice and barley belonging to the family Poaceae. The observations of each parameter were

mathematically averaged and then statistically analysed. The data are tabulated in tables 1 to 23 and presented in figures 1- 2 and plates 1 to 5. All plant crops showed their specific responses in terms of extraction of their biochemical components. However, their seeds were also noted for their percent germination.

No seed started germination till the first day (24 h) got passed. The seeds of *Avena sativa* continued germinating till day 4th. On the 2nd day the germination percentage was recorded as high as 73% in oat and as low as 57% in maize. Wheat, barley and rice showed germination percentage as 69.00, 61.00 and 59%, respectively.

On 3rd day recorded percent germination in wheat, oat, rice, barley and maize was 85.00, 89.00, 84.00, 86.00 and 79.00, respectively. Maximum germination percent on final day was recorded in oat (96) where as minimum value was recorded in maize (85) (Table 1). Besides this, the germination percentage in wheat, rice and barley was 91.00, 90.00 and 93.00, respectively. Germinated seeds were allowed to grow till 15 days.

Observations were also made on seedlings morphology, their fresh and dry weights in all five crop plants. These parameter showed differential pattern in different crops. The general pattern of change in length, fresh and dry weight of developing seeds, are shown in tables 2 to 6. The fresh and dry matter accumulation was observed on 3rd, 4th, 7th, 10th and 15th day of seedling stage.

There were found a regular increase in the length with the advancement of seedling age in all the five cultivars. The seedling length in wheat was recorded 3.14, 9.40, 14.50, 20.65 and 42.125 cm at 3rd, 5th, 7th, 10th and 15th day of plant age, respectively. The corresponding values for maize were 3.16, 10.73, 16.16, 30.25 and 80.16 cm. The length of seedlings in oat ranged from 3.65 cm (3rd day) to 53.65 cm (15th day). The respective values for barley were 2.72 cm and 40.10 cm. Minimum length of seedlings on 15th day was recorded in rice as 35.16 cm.

Fresh weight of seedlings showed an increasing trend with the advancement of their age in all the five cultivars. It measured 0.034, 0.214, 0.321, 0.825 and 2.218g in wheat and 0.092, 0.416, 0.617, 2.185 and 7.000 g in maize at a respective age of 3rd, 5th, 7th, 10th and 15th day. The corresponding values for oat and barley were 0.082, 0.433, 0.621, 0.917 and 2.468g and 0.038, 0.204, 0.318, 0.765 and 3.012g. Values for fresh weight of seedlings of rice were 0.021, 0.196, 0.315, 0.750 and 2.257g on 3rd, 5th, 7th, 10th and 15th day, respectively. Dry weight of seedlings in all the five cultivars was also measured at different ages. It measured 0.016, 0.032, 0.014, 0.036 and 0.007 g in wheat, oat, barley, maize and rice on the 3rd day of seedling age, respectively. The corresponding values for 7th and 15th day were 0.180, 0.314, 0.182, 0.717 and 0.180 g and 1.215, 1.416, 1.625,

2.895 and 1.416g, respectively. These observations show that minimum fresh and dry weight at 15th day was recorded in seedlings of wheat.

The data for chlorophyll content in seedlings of all the five cultivars have been set in tables 19-23. Amount of chlorophyll *a* in seedlings of wheat was recorded 1.336, 1.392, 1.498, 1.502 and 1.518 mg g^{-1} f.wt. on 3rd, 5th, 7th, 10th and 15th day old wheat seedlings plants and the corresponding values for rice and maize were 1.100, 1.316, 1.390, 1.392, 1.402 and 1.102, 1.316, 1.372, 1.378, 1.381 mg g^{-1} f.wt., respectively. In oat chlorophyll content ranged from 1.292 (3rd day) to 1.454 mg g^{-1} f.wt. (15th day). The respective values for barley were 1.312 and 1.482 mg g^{-1} f.wt. Minimum chlorophyll *b* content was observed in 3 day old seedlings of rice as 0.682 mg g^{-1} f.wt. while its maximum value was recorded in 15 day old seedlings of wheat as 0.821 mg g^{-1} f.wt. (Fig. 1).

On 15th day of seedling age the values of chlorophyll *b* recorded in rice, oat, maize and barley were 0.729, 0.765, 0.742 and 0.811 mg g^{-1} f.wt., respectively (Fig. 2). It is clear from observations that amount of chlorophyll *a* and *b* was found increasing with the advancement of the age of the seedlings in all the five crop species. The amount of total chlorophyll in 15day old seedlings was 2.339, 2.131, 2.123, 2.219 and 2.293 mg g^{-1} f.wt., respectively in wheat, maize, barley, oat and rice. It is also clear from the data obtained that amount of chlorophyll *a* is found higher than chlorophyll *b* at all the stages of seedling growth in all the five test seedlings plants. Slight increment was observed in chlorophyll *a/b* ratio from initial to the final stage of seedlings. In case of seedlings of wheat and rice at 3rd and 15th day chlorophyll *a/b* ratio was 1.744 and 1.848; 1.612 and 1.923, respectively. The corresponding values for maize and oat were 1.547 and 1.861; 1.814 and 1.900. Chlorophyll *a/b* ratio in barley was 1.768 in 3 day old seedlings and 2.249 in 15 day old seedlings. The maximum chlorophyll *a/b* ratio was recorded in 15 day old seedling of barley (Fig. 3).

Leaf extract pH did not show any specific pattern of increasement over the age and it ranged from 5.300 to 6.900 in all the cultivars. Maximum value for this parameter was recorded in 3 day old seedlings of oat (6.800).

Ascorbic acid content was also amounted in all the five cultivars at 3rd, 5th, 7th, 10th and 15th day of seedling stage. On 3rd day the values amounted to 0.244, 0.653, 0.519, 0.243 and 0.212 mg g^{-1} f.wt. in wheat, rice, maize, and barley, respectively (Table 19-23). The corresponding values for 7th day were 0.206, 0.623, 0.438, 0.203 and 0.165. In 15 day old seedlings of wheat, rice, maize, oat and barley amount of ascorbic acid was calculated as 0.216, 0.640, 0.502, 0.220 and 0.190 mg g^{-1} f.wt., respectively. The maximum amount of ascorbic acid on 15th day was recorded in seedlings of rice while at this stage its minimum value was amounted in barley.

Amount of carotenoid and anthocyanin was also calculated at each stage of seedlings in all the experimental plants. In wheat amount of carotenoid content was recorded 0.643, 0.681, 0.698 0.702 and 0.705 mg g^{-1} f.wt. at 3rd, 5th, 7th, 10th and 15th day of seedling age, respectively. The corresponding values for rice and maize were

0.405, 0.431, 0.448, 0.452 and 0.454 and 0.314, 0.335, 0.358, 0.360 and 0.362, respectively.

Amount of carotenoid content in oat and barley seedlings on 15th day was 0.647 and 0.692 mg g^{-1} f.wt., respectively. The results indicate an increasing trend in carotenoid content in seedlings of all the crops with advancement of crop age. Tables 19-23 are indicating the amount of anthocyanin in all the five cultivars at each stage of seedlings. The amount of anthocyanin recorded was, 0.098, 0.125, 0.102, 0.108 and 0.091 mg g^{-1} f.wt., in wheat, rice, maize, oat and barley at 3rd day, respectively. The corresponding values on 15th day were 0.147, 0.312, 0.351, 0.382 and 0.203. Maximum amount of anthocyanin was recorded in oat while its minimum value was recorded in wheat seedlings at this stage.

Protein and carbohydrate content were also calculated in the seeds and seedlings of the all five test seedlings plants. Result of protein analysis is shown in Tables 7-12. Amount of protein in seeds of wheat, rice, maize, barley oat and rice was recorded 45.000, 25.002, 38.005, 30.908 and 32.000 mg g^{-1} f.wt., respectively. On 3rd day of seedling stage protein was amounted to 80.762, 31.921, 65.452, 54.469 and 42.876 mg g^{-1} f.wt., in wheat, maize, barley, oat and rice, respectively. The value of this biochemical component was highest 80.762 mg g^{-1} f.wt. in wheat and

Some nutrients like nitrogen, phosphorus, calcium, iron, magnesium, potassium, zinc, sodium, copper and manganese were also amounted in the seeds and seedlings of different ages of all the five experimental crops (Tables 7-12). Amount of nitrogen in the seeds of wheat, rice, maize, barley and oat was recorded as 2.169, 1.152, 2.163, 1.324 and 1.934 mg g^{-1} f.wt, respectively. The biochemical component of nitrogen in 3 day old seedlings of wheat, maize, barley, oat and rice was 4.240, 4.627, 1.827, 1.445 and 1.824 mg g^{-1} f.wt, respectively. The highest value was found in maize 4.627 mg g^{-1} f.wt and lowest in oat 1.445 mg g^{-1} f.wt. The amount of nitrogen in 5 day old seedlings of wheat, maize, barley, oat and rice was 4.642, 4.719, 2.482, 1.627 and 2.118 mg g^{-1} f.wt, respectively. The highest values of nitrogen in 7 day old seedling of wheat, maize, barley, oat and rice were 4.749, 4.781, 2.998, 1.825 and 2.756 mg g^{-1} f.wt, respectively. The highest value was found in maize (4.781 mg g^{-1} f.wt) and lowest in oat (1.825 mg g^{-1} f.wt).

The amount of nitrogen observed in 10 day old seedlings of wheat, maize, barley, oat and rice was 5.584, 4.967, 3.062, 2.023, and 3.239 mg g^{-1} f.wt, respectively. The highest value was found in wheat 5.584 mg g^{-1} f.wt and lowest in oat 2.023 mg g^{-1} f.wt. The corresponding values for the same parameter on 15th day of seedling stage were 5.781, 3.718, 5.630, 3.951 2.730 and 3.718 mg g^{-1} f.wt of tested seedlings. The maximum amount of nitrogen was found in wheat seedlings and minimum in oat seedlings on 15th day (Fig. 6).

Phosphorous content amounted to 0.181, 0.064, 0.098, 0.037 and 0.047 mg g^{-1} f.wt. was noted in the seeds of wheat, rice, maize, barley and oat, respectively. The value of phosphorous in 3 day old seedlings of wheat,

maize, barley, oat and rice was 1.527, 3.263, 0.637, 1.212 and 0.429 mg g^{-1} f.wt, respectively. The maize seedlings were found to contain the highest value 3.263 mg g^{-1} f.wt and lowest value 0.429 mg g^{-1} f.wt was found in rice.

The content of phosphorous in 5 day old wheat, maize, barley, oat and rice seedling was 1.002, 2.096, 0.458, 0.894 and 0.312 mg g^{-1} f.wt, respectively. The maize plants had highest value 2.096 mg g^{-1} f.wt and lowest value 0.312 mg g^{-1} f.wt in rice. The values of phosphorous in 7 day old seedlings of wheat, maize, barley, oat and rice were 0.667, 1.656, 0.303, 0.529 and 0.189 mg g^{-1} f.wt, respectively.

Phosphorous content observed in 10 day old plant seedlings viz. wheat, maize, barley, oat and rice was 0.653, 1.592, 0.296, 0.517, and 0.179 mg g^{-1} f.wt, respectively. The maize plants were found to have highest value 1.592 mg g^{-1} f.wt and lowest value was found in rice 0.179 mg g^{-1} f.wt. The minimum and maximum values of phosphorous in 15 day old seedlings were recorded in rice 0.162 mg g^{-1} f.wt and maize 1.503 mg g^{-1} f.wt while it was amounted to 0.635, 0.285 and 0.509 mg g^{-1} f.wt in wheat, barley and oat (Fig. 7), respectively.

Calcium was amounted to 2.028, 0.829, 1.802, 1.324 and 1.761 mg g^{-1} f.wt in the seeds of wheat, rice, maize, barley and oat, respectively. The value of calcium in 3 day old seedling of wheat, maize, barley, oat and rice was 0.809, 0.690, 1.942, 3.243 and 0.885 mg g^{-1} f.wt respectively. Oat plants showed highest value 3.243 mg g^{-1} f.wt and wheat lowest value 0.809 mg g^{-1} f.wt. The calcium amounted in 5 day old seedling of wheat, maize, barley, oat and rice was 0.721, 0.518, 1.426, 2.819 and 0.625 mg g^{-1} f.wt, respectively. Oat plants showed highest value 2.819 mg g^{-1} f.wt. and wheat low value 0.518 mg g^{-1} f.wt. Data on yield of calcium in 7 day seedling plants viz. wheat, maize, barley, oat and rice were 0.491, 0.511, 1.260, 2.521 and 0.449 mg g^{-1} f.wt, respectively. Oat plant showed highest value 2.521 mg g^{-1} f.wt and rice lowest value 0.449 mg g^{-1} f.wt.

Calcium content estimated in 10 days old seedlings of wheat, maize, barley, oat and rice was 0.480, 0.496, 1.186, 2.216 and 0.434 mg g^{-1} f.wt, respectively (Fig. 8). Oat plants again showed highest value 2.216 mg g^{-1} f.wt and rice with lowest value 0.434 mg g^{-1} f.wt. This results got repeated in 15 d old seedlings also (Fig. 8).

Table 1 : Percent germination in seeds of wheat, maize, barley, oat and rice.

Day	Wheat	Maize	Barley	Oat	Rice

2	69.00	57. 00	61. 00	73. 00	59. 00
3	85. 00	79. 00	86. 00	89. 00	84. 00
4	91. 00	85. 00	93. 00	96. 00	90. 00

Table 2 : Length and weight of seedlings (3 days) in wheat, maize, barley, oat and rice.

Seedlings	**Wheat**	**Maize**	**Barley**	**Oat**	**Rice**
Fresh wt (g)	0.034 ±0.003	0.092 ±0.081	0.038 ±0.004	0.082 ±0.007	0.021 ±0.019
Dry wt (g)	0.016 ±0.002	0.036 ±0.06	0.014 ±0.002	0.032 ±0.005	0.007 ±0.001
Length (cm)	3.14 ±1.00	3.16 ±1.21	2.72 ±1.02	3.65 ±1.42	2.21 ±1.50

± Standard deviation.

Table 3 : Length and weight of seedlings (5 days) in wheat, maize, barley, oat and rice.

Seedlings	**Wheat**	**Maize**	**Barley**	**Oat**	**Rice**

Fresh wt (g)	0.214 ±0.009	0.416 ±0.018	0.204 ±0.008	0.433 ±0.019	0.196 ±0.007
Dry wt (g)	0.110 ±0.009	0.207 ±0.023	0.125 ±0.010	0.205 ±0.021	0.088 ±0.006
Length (cm)	9.400 ±1.168	10.730 ±1.000	8.660 ±0.433	10.720 ±1.000	6.780 ±0.861

± Standard deviation.

Table 4 : Length and weight of seedlings (7 days) in wheat, maize, barley, oat and rice.

Seedlings	**Wheat**	**Maize**	**Barley**	**Oat**	**Rice**
Fresh wt (g)	0.321 ±0.006	0.617 ±0.012	0.318 ±0.005	0.621 ±0.011	0.315 ±0.005
Dry wt (g)	0.180 ±0.004	0.717 ±0.012	0.182 ±0.004	0.314 ±0.007	0.180 ±0.004
Length (cm)	14.500 ±1.216	16.160 ±1.500	12.512 ±0.621	15.616 ±1.200	10.160 ±1.000

± Standard deviation.

Table 5 : Length and weight of seedlings (10 days) in wheat, maize, barley, oat and rice.

Seedlings	Wheat	Maize	Barley	Oat	Rice
Fresh wt (g)	0.825 ±0.006	2.185 ±0.131	0.765 ±0.013	0.917 ±0.009	0.750 ±0.069
Dry wt (g)	0.315 ±0.018	1.216 ±0.092	0.615 ±0.031	0.421 ±0.023	0.382 ±0.015
Length (cm)	20.652 ±1.020	30.251 ±0.716	18.168 ±1.210	22.811 ±0.521	18.159 ±1.420

± Standard deviation.

Table 6 : Length and weight of seedlings (15 days) in wheat, maize, barley, oat and rice.

Seedlings	Wheat	Maize	Barley	Oat	Rice
Fresh wt (g)	2.218 ±0.026	7.000 ±0.081	3.012 ±0.041	2.468 ±0.029	2.257 ±0.036
Dry wt (g)	1.215 ±0.013	2.895 ±0.021	1.625 ±0.019	1.416 ±0.016	1.416 ±0.017
Length (cm)	42.125 ±1.35	80.165 ±1.02	40.109 ±1.65	53.651 ±1.03	35.162 ±0.45

± Standard deviation.

DISCUSSION

The study on the extraction of certain biologically important chemicals from the seedlings of some selected plants viz. wheat, rice, maize, oat and barley was undertaken, firstly, with a view, to understand the biochemical changes and elucidate the mechanisms controlling the synthesis of various constituents in developing seeds into seedlings. The studies on the percent germination of all the five cereals viz. wheat, maize, barley, oat and rice were conducted. During all the three days of observation i.e. days 2, 3 and 4, respectively, maximum percent germination was observed with respect to oat whereas minimum percent germination was noticed for maize seeds. For oat the value observed for days 2, 3 and 4 were 73.00, 89.00 and 96.00 percent, respectively.

SUMMURY

the present study entitled study on nutritionally important biochemicals from the seedlings of some selected plants of the family *poaceae* (wheat, rice, maize, oat and barley) the effect of amala *(phyllanthus emblica)* fruit proteins extracts was studied on selected bacterial strains for antibacterial activity at different ph and solvents. the present finding of the experiments are presented in the followings tables and figures under appropriate headings.

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