

ORIGINAL ARTICLE

Effect of gibberellins and ascorbic acid treatment on phytic acid and micronutrients dialyzability in germinated biofortified wheat seeds

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Citation

Verma M, Bora RS, Sheikh Im Kumar V, Sangwan P, Dhaliwal HS. Effect of gibberellins and ascorbic acid treatment on phytic acid and micronutrients dialyzability in germinated biofortified wheat seeds. Indian J Comm Health. 2021;33(1):123-129. <https://doi.org/10.47203/IJCH.2021.v33i01.017>

Source of Funding: Nil Conflict of Interest: None declared

Article Cycle

Received: 05/03/2021; Revision: 15/03/2021; Accepted: 22/03/2021; Published: 31/03/2021

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Abstract

Introduction: Phytic acid chelate minerals, including Fe and Zn and render them inaccessible once ingested by human beings. The evaluation of differences in the dialyzability of macronutrients including Fe and Zn in various wheat derivatives is therefore important for the enhancement of nutritional quality of grains. **Objectives:** The objective of current study was to improve the micronutrient content in wheat grain. **Methods:** During germination (12, 24, 48, 72, 96 h), effects of gibberellins and ascorbic acid on phytic acid content as well as dialyzability of iron and zinc of wheat derivatives were determined. **Results:** The phytic acid content in wheat flour was determined and it was found 7.61 to 7.48 mg/g. After the treatment with gibberellins, it was significantly reduced from 8.68 to 21.6 % and 9.65 to 20.9 % with ascorbic acid. In wheat flour dialyzability of Fe was 4.53 to 8.97 mg/kg. After germination, Fe content was increased from 9.77 to 32.0 % with gibberellin, and 13.9 to 31.0 % with ascorbic acid. Moreover, with gibberellins, Zn content was increased from 8.68 to 21.6 % and 9.65 to 20.9 % with ascorbic acid, respectively. **Conclusion:** These results suggested that gibberellins as well as ascorbic acid can be exploited to improve the dialyzability of iron and zinc content due to reduced antinutrient i. e phytic acid and make the minerals available for the absorption in monogastric animals including human beings.

Keywords

Wheat Seeds; Gibberellins; Ascorbic Acid; Phytic Acid; Micronutrient Dialyzability

Introduction

Deficiencies of micronutrients in developed and developing countries affect approximately 50% of the global population. The most severe health constraints worldwide are iron, zinc and vitamin A deficiencies. (1) Micronutrients deficiencies affect the growth, reproduction, cognitive development and physical work ability and also increase the disease risk in children and adults, globally. (2) Phytic acid is known to chelate iron and zinc in cereals and is considered the major reasons for their poor bioavailability in humans. (3) Phytic acid is the primary phosphorus storage compound in seeds. (4) It has negative charge at physiological pH, which enables it to bind to positive charged compounds such as Mg^{2+} , Cu^{2+} , Zn^{2+} and Fe^{3+} . (5) Under physiologically essential conditions, the phosphate groups in phytic acid are negatively charged, leading to chelation of Fe and Zn, and thus rendering these minerals less accessible for absorption in gastrointestinal tract. (4,6) Some issues with single grain food supplement are that they contain inadequate amounts of essential micronutrients and the absorption of these micronutrients is often reduced by high level of antinutrients in most conventional diets. (7) Cereals not only have essential dietary ingredients, but some cereals might also enhance human health through their functional food attributes. (8) Legumes and cereals are the main food resources in developing nations that produce an abundant amount of phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakisphosphoric acid). However, the good news is that micronutrient deficiency such as Fe deficiency can be controlled by dietary enhancement, supplementation and fortification. (9) Fe and Zn deficiency has been identified as public health problem and estimated that about two billion people worldwide suffer from micronutrient related disease, 30 % of which are anaemic, while 17.3 % suffer from insufficient Zn intake. (10)

Biofortification, one of the essential sustainability solutions proposed by the World Health Organization (WHO), has been implemented in many countries to accomplish the targets of controlling micronutrient deficiencies. (11) Depending on the plant species and the micronutrient type, different approaches for biofortifying plants with micronutrients are employed. The micronutrients are transported from the soil to the plant. There is

also a strong genotype and environment (G×E) interaction with regards to iron and zinc uptake.

Several genes are involved in absorption, translocation and loading as these nutrients are passed from soil to crop. In comparison with the wheat cultivars, the wheat-Aegilops replacement lines of groups 2 and 7 chromosomes were previously developed showing high grain Fe and Zn. (12) Highly stable BC2F9 wheat–Aegilops substitution lines have been developed in the farm of Eternal University, Baru Sahib, Himachal Pradesh, with the recommended fertilizers, urea and ammonium phosphate. (13) Biofortified wheat genotypes with enhanced mineral content and quality attributes have recently been reported. (14) Various methods have been employed to remove phytic acid from grains. (15) Removal of phytic acid from food and feed will improve their nutritional value and increase micronutrient bioavailability, apart from mitigating environmental pollution caused by high phosphorous content in soil. The analysis of this study was to evaluate the effect of gibberellins and ascorbic acid on phytic acid as well as dialyzability of iron and zinc content in biofortified wheat derivatives.

Aims & Objectives

1. Effect of gibberellins and ascorbic acid treatment on phytic acid content
2. Effect of gibberellins and ascorbic acid treatment on micronutrients (Fe and Zn) dialyzability and availability

Material & Methods

Plant material: Biofortified wheat derivatives have been obtained from the department of Genetics, Plant breeding & Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture Eternal University, Baru Sahib, Himachal Pradesh. These selected wheat derivatives are MB-64-1-1, 49-1-11-9-7-1 and one control wheat variety i.e. PBW343+GPC+Lr24 (PBW343Lrp).

Wheat seeds germination under the influence of gibberellins and ascorbic acid: During soaking of wheat seeds, gibberellin was added at concentrations of 1 g/L and ascorbic acid 2 g/L and allowed to germinate for different time duration i.e. 12, 24, 48, 72, 96 h at 25 °C. The seeds were washed with distilled water once a day during the germination period. After germination, seeds were collected and dried in oven at 45 °C for analysis of

phytic acid and micronutrients dialyzability (i.e iron and Zinc).

Estimation of phytic acid contents affected by germination in biofortified wheat derivatives:

The extraction and quantification of phytic acid in the biofortified wheat derivatives was evaluated by Gao et al. 2007 [16], with minor modifications. Phytic acid extraction from wheat flour (0.25 g) was carried out in 10 mL of 2.4% HCl in a 15 ml falcon tube. The mixture was vortexed and placed at room temperature for 16 h overnight. The samples were then kept in shaking condition for 2 h at 200 rpm. Following this, sample mixture was centrifuged at 8000 rpm for 15 min. For estimation of phytic acid content, 1800 µl supernatant was taken in a test tube and 600 µl of freshly prepared Wade reagent (0.09 g Sulpho salicylic acid and 0.009 g ferric chloride into 30 mL distilled water) was added. The samples were incubated for 20 min and the absorbance was measured at λ500 nm against the reference control. In order to measure the actual absorbance, the values were deducted from the absorbance of blank. The standard curve was prepared using phytic acid (sodium salt) at the concentration of 0, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 µl. Quantification of phytic acid (mg/g) was performed using a standard curve

Estimation of dialyzable micronutrients as affected by germination in biofortified wheat derivatives:

Dialyzability of iron and zinc was determined according to method as described by Miller et al. 1981. (17) For analyzing the dialyzability of iron and zinc, 1 g of wheat flour was weighed and 10 mL sodium acetate buffer (0.1 M, pH 5.0) was added and incubated at 25 °C for 2 h in incubator shaker at 200 rpm. The sample was then transferred into the pre-activated dialysis membrane (cut off 12 kDa) bag. The dialysis bag was kept in 50 mL of NaCl solution (10 mg/mL). The solution was dialyzed at room temperature (i.e. 25°C) for 2 h. solution was replaced with fresh NaCl solution twice and kept for overnight. The solutions were pooled and amount of dialyzable Fe, Zn contents in this solution was measured by atomic absorption spectrophotometer.

Statistics: The experiments were carried out in triplicates and the data presented as mean ± standard deviation. The standard deviation of average was calculated by Microsoft office Excel 2010 and graph was prepared by using graph prism 5. [Group of mean values obtained from Tukey Test by using one way ANOVA. Different wheat

derivatives group with different letter in common (a, b, c, d, e, f, g) differ significantly from each other for the variable listed (Tukey Test P<0.05)]

Results

Effect of gibberellins and ascorbic acid on phytic acid content in the germinating biofortified wheat seeds:

Effect of activators such as gibberellins and ascorbic acid on phytic acid content was analysed in the wheat derivatives as shown in (Figure 1). The phytic acid content in untreated sample was evaluated and it was found to be 8.08 in control wheat cultivar PBW343Lrp, 7.48 mg/g in biofortified wheat derivatives 49-1-11-9-7-1 and 7.61 mg/g in MB-64-1-1 after 12 h germination. The phytic acid content in derivatives was reduced from 21.9 to 27.3 % in the presence of gibberellin and 21.8 to 28.1 % of initial value with ascorbic acid treatment after 12 h germination. After germination for 24 h, phytic acid was reduced in the range of 20.2 to 24.5 % with gibberellin and it was reduced upto 23.5 to 25.5 % of initial value after ascorbic acid treatment. During 48 to 96 h post germination, it was found to decrease in the range of 31.1 to 34.8 % with gibberellin and 30.9 to 35.0 % of initial value with ascorbic acid respectively, as compared to control sample.

Effect of gibberellins and ascorbic acid on dialyzability of iron in the germinating biofortified wheat seeds:

The effect of gibberellin and ascorbic acid during germination on the dialyzability of Fe is presented in (Figure 2). Germination in the presence of gibberellin and ascorbic acid enhanced the dialyzable Fe content in selected wheat derivatives. In wheat flour dialyzable Fe content was 4.53 to 8.97 mg/kg and after 12 h germination with gibberellin treatment, dialyzable Fe was increased from 42.6 to 51.6 %, while with ascorbic acid it was increased 44.2 to 50.8 % of initial value. After 24 h germination, Fe content was increased 50.7 to 63.8 %, however after ascorbic acid treatment for 24 h germination, dialyzable Fe was increased from 51.7 to 63.8 % of initial value as compared to untreated sample. During 48 to 96 h post germination, Fe content was increased in the range 73.5 to 111.4 %, while with ascorbic acid it was increased in range of 95.2 to 109.8 % of initial value. With activators treatment, maximum dialyzable Fe content was increased in derivative MB-64-1-1 by 111.4 %, respectively.

Effect of gibberellins and ascorbic acid on dialyzability of zinc in the germinating biofortified wheat seeds:

The effect of activators on dialyzability

of zinc was analysed in different wheat derivatives and data is shown in (Figure 3). Similar to Fe, the dialyzability of Zn was also increased after treatment with activators. In wheat flour dialyzable Zn content was 4.57 to 7.06 mg/kg least dialyzable Zn was observed in control non-biofortified wheat cultivar PBW343Lrp i.e. 4.57 mg/Kg. After 12 h germination, dialyzable Zn content was increased 7.13 to 14.4 %, with gibberellins and 9.53 to 15.4 % increase with ascorbic acid treatment. During 24 h germination in the presence of gibberellins, Zn content was increased in range of 39.0 to 41.9 %, while with ascorbic acid it was increased from 40.3 to 62.6 % of initial value. After 48 to 96 h post germination, Zn content was increased maximum in the range of 53.5 to 70.8 % with gibberellins and it was found to increase upto 79.1 to 107.8 % of initial value, with ascorbic acid, respectively. Overall, dialyzable zinc content was increased with activators treatment. In control wheat cultivar PBW343Lrp, minimum increase of dialyzable Zn content was observed in comparison to other biofortified wheat derivatives. In wheat derivatives, treatment with activators i.e gibberellins and ascorbic acid, led to significantly higher Zn dialyzability as compared to control wheat.

Discussion

The results of our analysis of phytic acid analysis showed that gibberellins and ascorbic acid treatment resulted in enhanced phytic acid degradation during germination. The content of phytic acid was reduced by 31.1 to 34.8 % with gibberellins and 30.9 to 35.0 % with ascorbic acid, respectively. This hormonal effect can be experimentally manipulated by soaking seeds with GA₃, which is essential for seed germination and the endosperm degradation. (18) In similar studies, after gibberellins treatment, phytic acid was significantly decreased from 93.28 % during 4th days of germination as compared with 0 days (19). Phytic acid was reduced by adding ascorbic acid rich food like tomatoes, kale that increased the dialyzability and availability of micronutrients (20-22).

In our experiment, the treatment with gibberellins and ascorbic acid led to improve the dialyzability of iron as well as zinc content in selected biofortified wheat derivatives. The treatment with ascorbic acid provides an effective mean to prevent iron deficiency in human. (23,24,25) Ascorbic acid has long been known to improve the iron absorption in cereal based food. (26) In both animals and plants,

ascorbic acid has many major biological roles. (27,28) The germination process with ascorbic acid has been reported to have a significant effect to increase nutrients in soybeans. (29)

In order to mitigate the inhibitory effect of phytic acid on zinc and iron absorption, the addition of organic acid, in particular ascorbic acid and other complexing agents have been employed. (30) The addition of ascorbic acid (5 mg or 10 mg) substantially enhanced Zn content in chick peas from 6.54 to 7.94 mg/100g, while it improved from 9.19 to 11.00 mg/100g in red kidney beans. In green gram, Zn content increased from 9.69 to 11.71 mg/100g after the treatment with ascorbic acid. In an earlier study, Zn content indicated a small increase in the presence of ascorbic acid in bread. There was no effect on the absorption of Zn at a low dose of ascorbic acid in high fiber diets such as whole meal bread. (31) The variability in Zn and Fe bioavailability can occur due to varying food matrix and processing type. (32) The ascorbic acid had been shown to enhance Fe and Zn absorption. (31) High phytic acid present in foods inhibits Fe and Zn absorption. (33) However, ascorbic acid decreases the inhibitory effect of phytic acid on Fe absorption. (34)

Conclusion

The grain micronutrients dialyzability of iron and zinc in biofortified wheat-Aegilops derivatives was analyzed during gibberellins and ascorbic acid treatment. Germination with gibberellins as well as ascorbic acid treatment significantly reduced the phytic acid content and increased micronutrient dialyzability. Our study clearly indicates that wheat seeds are useful sources of micronutrients during germination and treatment with activators such as gibberellins and ascorbic acid, could be used to degrade antinutrient i.e. phytic acid in cereals based food, to enhance the dialyzability of micronutrients. The enhancement of the nutritional quality will help the consumers to obtain better access to the health benefits of wheat.

Abbreviations used

G: germination; WS: wheat seeds; Fe: iron; Zn: zinc.

Recommendation

The daily recommended diet of food does not provide the sufficient amount of Fe as well as Zn and causes many deficiency diseases in humans. The improvement of these micronutrients during germination after treatment with gibberellins and

ascorbic acid can be very helpful to overcome the problem of malnutrition in children and adults in developing countries.

Relevance of the study

Gibberellins and ascorbic acid treatment in germinated cereals grains is very useful for degradation of phytic acid and to enhance the micronutrients (Fe and Zn) dialyzability in monogastric animals and human.

Authors Contribution

MV: Acquisition and data analysis, RSB: Interpretation and data drafting, IS: Data analysis, VK: Substantial contribution to conception and design, PS: Data Analysis, HSD: Substantial contribution to conception and design.

Acknowledgement

We acknowledge the financial support provided by Department of Biotechnology (DBT), Govt. of India (Grant No. BT/AGR/BIOFORTI/PHII/NIN/2011) to conduct the part of this study and Ministry of Food Processing Industries (MoFPI), Govt. of India for infrastructure facility development at Eternal University, Baru Sahib.

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Figures

FIGURE 1 EFFECT OF GIBBERELLINS AND ASCORBIC ACID TREATMENT ON PHYTIC ACID CONTENT IN GERMINATED WHEAT SEEDS [DIFFERENT WHEAT DERIVATIVES GROUP WITH DIFFERENT LETTER IN COMMON (A, B, C, D, E, F) DIFFER SIGNIFICANTLY FROM EACH OTHER FOR THE VARIABLE LISTED (TUKEY TEST P<0.05)]

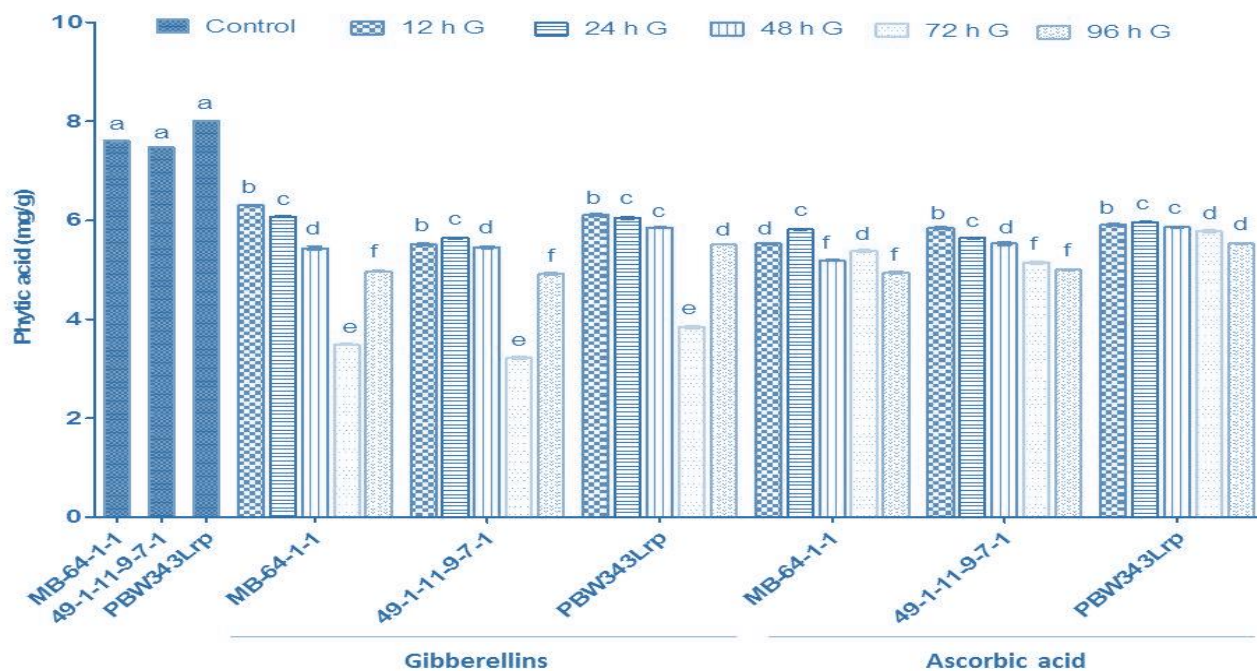


FIGURE 2 EFFECT OF GIBBERELLINS AND ASCORBIC ACID TREATMENT ON DIALYZABILITY OF IRON CONTENT IN GERMINATED WHEAT SEEDS [NO SIGNIFICANT DIFFERENCE BETWEEN SAME LETTERS (A, B, C, D, E, F, G) OF ENTRY FROM EACH OTHER FOR THE VARIABLE LISTED (TUKEY TEST P<0.05)]

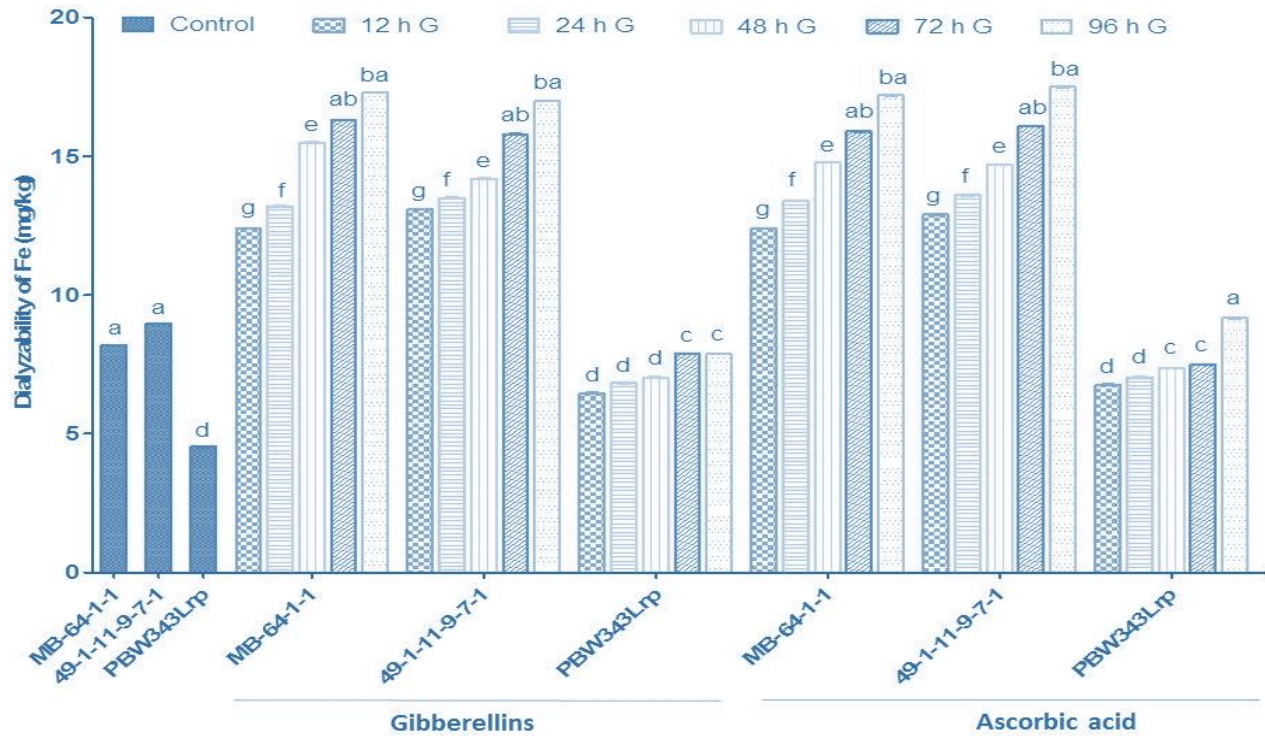


FIGURE 3 EFFECT OF GIBBERELLINS AND ASCORBIC ACID TREATMENT ON DIALYZABILITY OF ZINC CONTENT IN GERMINATED WHEAT SEEDS [DIFFERENT WHEAT DERIVATIVES GROUP WITH DIFFERENT LETTER IN COMMON (A, B, C, D, E, F) DIFFER SIGNIFICANTLY FROM EACH OTHER FOR THE VARIABLE LISTED (TUKEY TEST P<0.05)]

