moisture and 5% minerals. Soy protein is the best plant protein and its quality is equivalent to that of livestock proteins in terms of protein digestibility corrected amino acids score (PDCAAS) and it is least expensive (Rs 100–150/kg of soy protein, whereas pulse proteins cost Rs 300–350/kg of protein and that from livestock sources is Rs 500–1800/kg of protein)[3–6]. As of now the world soybean production is about 240 MT (million tonne) and that of India is about 13 MT. The five major soybean-producing countries of the world are USA, Brazil, Argentina, China and India[7,8].

One kilogram of dry soybean yields 4–6 litres of milk containing about 90% water, 3.5% protein, 2% fat and 4.5% non-lactose carbohydrate and phytochemicals. Special features of soymilk are low cost (Rs 15–20/litre), good nutrients and its suitability to lactose-intolerant people. It is the base material for making soy paneer (tofu), soy yoghurt and other dairy analogies. Soymilk can be used for feeding infants and as supplements to diets of the pre-school children, young adults and old people[9,10].

Soymilk has a characteristic beany flavour which may not be acceptable to all consumers[10–12]. The flavour can be minimized/masked using food flavour like cardamom, ginger, fruits (mango, apple, litchi, etc.) and chocolates while making soymilk as a beverage. The flavoured soymilk when served chilled found to be popular in countries where soymilk has been introduced recently[8].

The beany flavour in soymilk is on account of the presence of lipoxygenase enzymes in soybean which become active as soon as the cotyledons are broken and enzymes come in contact with water, oxygen and lipids; these combine together to give the beany flavour[13–19] as follows:

$H_2O + O_2$ + lipids + lipoxygenase $\rightarrow$
Beany flavour causing volatiles such as methanol, acetaldehyde, ethanol, hexanol, hexanal, pentanol, etc.

Lipoxygenase is heat-sensitive and gets inactivated at higher temperatures. Traditionally beany flavour in soymilk has been controlled/minimized by inactivating the enzymes through heating, or altering the pH of the aqueous medium in which soybean seeds are disintegrated[14,20–22]. Beany flavour in soymilk can be tackled by partially inactivating the enzyme and then removing the remaining flavour by deodorization and masking of the residual flavour by natural flavouring agents. Vacuum flash deodorizing system is a technically advanced device for removing/minimizing flavour from slurry or liquid food products[23–25]. The major advantage of this device is that it is a continuous operation, yields an excellent neutral/bland product and the level/degree of deodorization can be controlled[8,10]. The present communication discusses various methods of minimizing beany flavour is soymilk and the comparative performances in terms of physical, biochemical and microbiological aspects and suggests how to prepare soymilk with least beany flavour, good nutrition and low cost.

Soybean variety JS-335 grown at the farm of Central Institute of Agricultural Engineering, Bhopal was used for the study. The harvested soybean was cleaned, dried and stored in plastic containers at 4°C until it was processed for making soymilk.

Six different methods, namely traditional oriental method, hot-water grinding method, Illinois' method, rapid hydration hydrothermal cooking method, airless grinding method, and deodorization method were used for the production of soymilk (Figure 1). A brief description of the different processes is as follows:

Traditional oriental method (TOM): Soybean was soaked at room temperature for 4 h was ground and cooked. Cooking of soy-slurry/pulp with added water (6.0 kg/kg of dry soybean) was done for 20 min at 82°C. This was followed by filtering of cooked slurry using two-layer muslin cloth to separate out okara. This process is also known as cold-water grinding method.

Hot-water grinding method (HWGM): Soybean was soaked at room temperature for 4 h and then ground using boiling water to maintain soy slurry temperature of 90°C or more. This was followed by cooking for 15 min at 90°C or above and then filtering with two-layer muslin cloth to separate out soymilk and okara. This process reduces the beany flavour by inactivating lipoxygenases during grinding at 90°C and above.

Illinois method (IM): This method was developed at the University of Illinois at Urbana Champaign, USA. Soybean was first soaked for 12 h in 0.5% $NaHCO_3$ solution,

Figure 1. Soymilk prepared using different methods. TOM, Traditional oriental method; HWGM, Hot-water grinding method; IM, Illinois method; RHHM, Rapid hydration hydrothermal method; AGM, Airless grinding method and DM, Deodorization method.

drained and then boiled in fresh 0.5% $NaHCO_3$ solution for 30 min, drained again and ground in water at room temperature. The soy-slurry was then heated to 90°C and filtered to remove okara. The soymilk thus produced has bland flavour.

Rapid hydration hydrothermal method (RHHM): Soybean was ground into flour and made into slurry using hot water and then pressure-cooked for 30 sec, cooled and adjusted to 9% solid with water and centrifuged to get the soymilk.

Airless grinding method (AGM): Soybean was soaked for 4 h at room temperature and ground under airless condition at 120°C for 20 min, cooked and then filtered using two-layer muslin cloth to separate out milk and okara.

Deodorization method: A spiral-type soymilk deodorizer of 100 l/h was developed. The principle of the deodorizer is that under partial vacuum soymilk is passed through the cylindrical drum mounted with spiral plate assembly for the flow of milk to have maximum retention time and exposure surface area of milk during its flow for the production of more volatiles and sucking of these off-flavour causing components. The deodorizer consists of a cylindrical drum, spiral channel assembly, vacuum system, soymilk inlet system, feeding dome and outlet cone. The minimum length to diameter ration ($L:D$) of the cylindrical system is 2.8 : 1 for efficient discharge of fume. In the flow zone, 5 mm holes in two rows of spiral plate connected with pipes are provided for splashing of milk and initiation of further production of volatiles. The developed deodorizer was fitted to the existing mini soymilk plant (Figure 2). Soymilk prepared from traditional oriental method and hot water grinding method was used for the deodorization study. The deodorized soymilk was taken for quality analysis and sensory evaluation.

Figure 2. Testing the developed deodorizer with soymilk plant.

Soymilk produced using soybean : water ratio 1 : 6 by the above six different methods was analysed for is physico-chemical, proximate, hydroperoxide, antioxidant activity, lipoxygenase visual assay, microbiological qualities and sensory parameters using standard procedures. Various quality parameters of soymilk and procedures used for their determination are as follows:

Soymilk yield: This was determined by weighing the soybean slurry obtained immediately after wet grinding and soymilk obtained after filtration. Soymilk yield is expressed as the ratio of soymilk and slurry.

Physical characteristics: Solid content, pH value and conductometric analysis of soymilk were done using standard techniques and procedures.

Proximate analysis: Protein, fat, carbohydrate and ash contents of soybean and soymilk were determined in triplicate using standard method[26].

Hydroperoxide: This was determined using thiobarbituric acid reactive species assay[27].

Antioxidant activity (2,2-diphenyl-1-picrylhydrazyl (DPPH) assay): It is a stable free-radical in a methanolic solution. In its oxidized form, the DPPH radical has an absorbance maximum at about 520 nm (ref. 28).

Lipoxygenase activity: Lipoxygenase-catalysed oxidation, as well as chemical oxidation, may also be indirectly responsible for the formation of rancid off-flavour. The initial enzymatic reaction catalysed by lipoxygenase only involves the activation of substrate and incorporation of O_2 to form hydroperoxide. A visual procedure for the MBB method was modified from Suda *et al.*[29].

MBB reagent: The dye substrate was prepared by first weighing 154.25 mg of dithiothreitol and transferring it to a 125 ml flask. The following additional components were then added to the flask: 25.0 ml 0.2 M sodium phosphate buffer (pH 7.0), 5.0 ml 0.1 millimolar (mM) methylene blue, 5.0 ml acetone and 9.0 ml 0.01 M linoleic acid substrate. After adding all the components, the mixture was swirled. This dye substrate was adequate for 20 samples.

Procedure: First 10 ml soymilk sample was taken in the centrifugal tube and centrifuged at 10,000 *g* for 15 min for collection of supernatant. The assay was carried out by the mixing 0.6 ml supernatant and 2.0 ml MBB reagent in a clean test tube. A positive test was indicated by blue colour and score with respect to disappearance (bleaching) of blue colour within 2 min.

Total bacterial count: The soymilk samples were placed in a polypropylene container. Nutrient agar was used as the growth medium; it was prepared according to manufacturer's specifications. The standard tenfold serial dilution technique was employed to dilute each soymilk sample up to ten levels. The plates were then inoculated with 1 ml of the ten dilutions and then incubated at 37°C for 24 h, after which the number of viable cells was counted using a digital colony counter. All plates were triplicated, incubated at 37°C for 48 h, and viable cell numbers were determined as colony forming units (CFU) per ml.

Shelf life: Soymilk prepared using different methods was kept in an airtight container and stored at two different conditions for shelf-life studies. One group was stored at room temperature (23° ± 2°C) and the other at refrigeration temperature (4°C). Shelf-life studies were carried out immediately after preparation (0 h) and every 12 h thereafter, until it was spoiled. Bad odour, bad taste and separation of soymilk for its shelf life were considered based on microbial population.

Organoleptic evaluation: Soymilk was evaluated for taste, texture, flavour, odour, appearance and overall acceptability by a panel of nine trained judges using a nine-point hedonic scale. The mean score of 5 was considered as acceptable. The response was critically analysed[30].

An experiment was conducted to study the effect of different methods used for preparation of soymilk and their effect on physio-chemical parameters, proximate composition, total bacterial count, hydroperoxide level and antioxidant activity. Completely randomized design (CRD) was used in the experiment. The experimental data were analysed using GLM procedure available in the statistical analysis software. The significance of different methods, their pairwise comparison and estimation of coefficients were carried out. The pairwise comparison was performed using Tukey's Studentized Range Test.

The physio-chemical study of soymilk quality obtained from six different processes indicates variation in solid yield content (%), protein yield, conductivity (mS), total dissolve solid (ppm) and pH (Table 1). The solid content of soymilk for all the samples varied from 5% to 8%. The highest solid content was in soymilk prepared by traditional oriental method and it could be a result of less solubilization at the time of grinding. The highest pH (7.39) was found in the Illinois method, which may be attributed to soaking and cooking of soybean in the presence of sodium bicarbonate. It is beneficial for inactivation of lipoxygenase enzyme and trypsin inhibitor activity at higher pH. The lipoxygenase activity and trypsin inhibitor activity were found to decrease with increase in temperature and pH. Highest protein yield (93%) was observed in soymilk prepared by Illinois method. A comparative study of different biochemical parameters in different methods indicates that the highest solid content is observed in traditional oriental method and lowest in Illinois method, and the conductivity of Illinois method was found to be maximum because of higher solubilization of solid and formation of higher active particles. Higher value of total dissolved solid was found in traditional oriental method, compared to other methods, and the protein yield was minimum in this method. Protein yield was maximum (92–93%) for the Illinois method and rapid hydration hydrothermal method. The protein extraction was higher in IM and RHHM due to higher membrane protein solubilization during high pressure and soaking in the presence of sodium bicarbonate. In the case of traditional oriental method, the protein yield was low due to room temperature grinding not resulting in much solubilization and low rate of extraction.

The proximate composition of soybean seeds used for the preparation of soymilk was moisture content (6.17%), protein content (34.51%), fat (17.09%), ash content (5.65%) and carbohydrate (36.58%). Table 2 shows the proximate composition of soymilk sample prepared by different methods. The moisture content of different soymilk samples was found to be in the range 89–92%. The protein content ranged from 2.81% to 3.19%. The protein content of IM, AGM and DM was higher in comparison to other methods. The fat content was found to be vary from 2.12% to 2.46%. The carbohydrate content of IM was lower compared to other methods due to longer time of soaking and cooking at several steps, which hydrolysed the carbohydrate, especially soluble sugars. Similar result was also reported by Wilkens *et al.*[31] and Shurpalekar *et al.*[32].

The soymilk prepared by traditional oriental method has strong beany flavour in comparison to soymilk prepared by other methods. In case of TOM, the formation of hydroperoxide in the presence of polyunsaturated fatty acid, air, water and lipoxygenase enzyme resulted in a conducive atmosphere for the production of higher hydroperoxide.

The more the production of hydroperoxide, higher is the level of beany flavour. The level of beany flavour and flavour quality was found to improve in soymilk prepared by IM, RHHM, AGM and DM. The lowest level of beany flavour compounds in these methods indicates lower level of hydroperoxides and reduced activity of lipoxygenase enzymes. The hydroperoxide content of normal soybeans (those with lipoxygenase activity) ranged from 63.5 to 83.6 nmol/g. The hydroperoxide content of soymilk showed variation with soymilk preparation metods. The hydroperoxide content of soymilk produced from different methods ranged from 4.73 to 17.50 nmol/g. Results obtained in this study are similar to those found in the literature[18,33]. It has been reported that the *n*-hexanal content, the major constituent of the beany flavour of soymilk, is directly proportional to that of hydroperoxide[33]. The beany flavour of soymilk prepared by all lipoxygenase-lacking soybeans is less than that of soymilk made from normal soybeans[34]. It is found that soymilk

Table 1. Physio-chemical analysis of soymilk prepared using different methods

Method of soymilk preparation	Solid yield (%)	Protein yield (%)	Solid contents (%)	Conductivity (mS)	TDS (ppm)	pH
Traditional oriental method	61	72	8.0	3.03	1520	6.52
Hot-water grinding method	65	82	7.4	1.54	776	6.75
Illinois method	88	93	5.2	3.23	1620	7.39
Rapid hydration hydrothermal method	86	89	6.8	2.93	1453	6.29
Airless grinding method	76	87	7.2	2.73	1372	6.31
Deodorization method	77	85	7.4	2.63	1357	6.40

Table 2. Proximate composition of soybean and soymilk prepared using different methods

Method of soymilk preparation	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrates (%)
Traditional oriental method	89.20	2.81	2.15	0.41	3.43
Hot-water grinding method	90.50	2.93	2.23	0.39	3.95
Illinois method	91.51	3.19	2.36	0.49	2.46
Rapid hydration hydrothermal method	91.16	2.94	2.39	0.42	3.15
Airless grinding method	90.50	3.12	2.27	0.33	3.78
Deodorization method	91.15	2.98	2.19	0.33	3.35

with minimal beany flavour can be prepared if the hydroperoxide content is approximately 5 nmol/g.

The hydroperoxide content in Illinois method and deodorization method was lower in comparison to other methods. The hydroperoxide content of soymilk in the control group was 17.5 nmol/g. The hydroperoxide content of heat-treated and sodium bicarbonate-treated soybean in the Illinois method was appreciably lower. The airless grinding method also showed lower levels of hydroperoxide in comparison to grinding with mixer-grinder. The soaking timing and duration of grinding in the absence of air were found to be more effective in reducing hydroperoxide in soymilk. Based on the above results, it can be inferred that if normal soybeans are blanched in boiling water after being soaked and swollen, the hydroperoxide content can be reduced without markedly diminishing the content of protein or solid matter, thus reducing the beany flavour[10,35]. There was 73.6% reduction in hydroperoxide content of soymilk prepared from deodorization method compared to traditional oriental method. However, 68% reduction of hydroperoxide was observed in soymilk prepared by Illinois method. Deodorization method is a chemical-free technique for minimization of beany flavour, resulting in higher acceptability. The antioxidant activity of soymilk samples was found maximum in traditional oriental method in comparison to other methods. This may be attributed to processing techniques used for soymilk preparation. In the traditional method, soymilk is prepared by grinding at room temperature resulting in minimum loss of antioxidants. However, in other methods either heat, chemical or pressure treatment is responsible for higher loss of antioxidants, giving lower values.

The lipoxygenase activity was found in all selected processes for soymilk preparation; it was higher in traditional oriental method and lower in deodorization and Illinois methods. The lox visual assay was determined to observe the disappearance of dye colour in soymilk sample. The lesser is the time required for colour disappearance indicates higher lipoxygenase activity. The colour disappearance is measure in 1 to 6 min for visual assay that is achieved in traditional method indicating higher lox activity. However, the colour disappearance in other methods took more than 30 min, suggesting lower activity of lipoxygenase enzyme and unfavourable conditions for the production of hydroperoxide. The percentage of original MBB activity remaining in the samples decreased with blanching time. Toyosaki[36] proposed a mechanism for the MBB reactions. Methylene blue was hypothesized to be bleached when the hydroperoxide was produced by removal of hydrogen. Suda *et al.*[29] also proposed that a redox reaction was involved in the bleaching reaction, because methylene blue is a redox indicator.

Raw soymilk and pasteurized soymilk were used for microbial load counting. Table 3 shows the results of microbial examination of soymilk prepared using the six methods. Maximum plate count was observed in using raw soymilk traditional oriental method because of less control measured during the production process. However, lower microbial count in other methods, especially IM and RHHM indicates unfavourable condition for microbial growth (Table 3). In pasteurised milk no bacterial count was observed in soymilk prepared from various methods and storage at 4°C (ref. 37).

In shelf-life studies, all samples except those traditional oriental method remained stable emulsion for both raw and pasteurized conditions. The beany flavour of raw soymilk (TOM) became stronger during the second day of storage at 4°C. By the fourth day of storage, the odour of other samples became stronger and moulds had started

Table 3. Total bacterial count of raw and pasteurized soymilk using different methods

Method of soymilk preparation	Raw soymilk (cfu/ml)	Pasteurized soymilk (cfu/ml)*
Traditional oriental method	9.60×10^5	No bacterial growth
Hot-water grinding method	7.60×10^5	No bacterial growth
Illinois method	3.80×10^5	No bacterial growth
Rapid hydration hydrothermal method	5.30×10^5	No bacterial growth
Airless grinding method	4.13×10^5	No bacterial growth
Deodorization method	4.11×10^5	No bacterial growth

*Analysis was performed after 7 days of storage at 4°C.

Table 4. Sensory evaluation of soymilk samples

Method of soymilk preparation	Appearance	Flavour	Taste	Overall acceptability	Beany flavour	Lox visual assay test (1–6 min)
Traditional oriental method	6.3	5.5	6.0	6.0	Strong	7
Hot-water grinding method	7.2	6.25	6.76	6.50	Improved	NA
Illinois method	7.5	7.29	7.35	7.30	Least	NA
Rapid hydration hydrothermal method	6.5	6.19	6.9	6.70	Less	NA
Airless grinding method	7.0	6.72	7.12	6.55	Less	NA
Deodorization nethod	7.7	7.44	7.50	7.50	Least	NA

Table 5. ANOVA of hydroperoxide level and antioxidant activity of soymilk prepared using different methods

Source	Degree of freedom	*P* value	
		Hydroperoxide	Antioxidant
Model	5	<0.0001	<0.0001
Traditional oriental method		<0.0001	<0.0001
Hot-water grinding method		<0.0001	0.02
Illinois method		<0.0001	0.0001
Rapid hydration hydrothermal method		<0.0001	<0.0001
Airless grinding method		<0.0001	<0.0001
Deodorization method		<0.0001	0.0001
Error	12		
Total	17		
R^2		0.99	0.84
CV		1.01	7.54

Table 6. Pairwise comparison of hydroperoxide level and antioxidant activity of soymilk prepared using different methods

Method	Means	
	Hydroperoxide	Antioxidant
Traditional oriental method	17.61	41.66
Hot-water grinding method	10.34	36.33
Illinois method	5.61	30.66
Rapid hydration hydrothermal method	6.77	28.66
Airless grinding method	7.44	29.00
Deodorization method	4.76	30.33
Mean significance difference	0.24	6.78

to grow. The pasteurized and refrigerated soymilk had no change in taste and odour after one week of storage, and when the samples were examined microbiologically on the sixth day, there was no growth. This indicates that the pasteurization temperature and time are adequate for the packaging and storing of soymilk, regardless of the production method.

The sensory quality attributes (appearance, flavour, taste and overall acceptability) of soymilk samples were evaluated using nine-point hedonic scale (Table 4). It was found that the sensory quality of traditional soymilk was just good. However, the quality parameters as well as the overall acceptability of soymilk prepared from deodorization and Illinois methods was very good, as indicated from the sensory score data. The sensory analysis indicated that soymilk prepared using Illinois and deodorization methods was found to be creamy, with bland taste, least odour and no crystalline solids. The sensory quality of soymilk prepared using Illinois method indicated that sodium bicarbonate not only had an effect of softening the soybean, but also removed the beany flavour and left the soymilk blend. The deodorized soymilk operated at partial vacuum, sucked and removed the volatile compounds responsible for beany flavour without the use of any chemical treatment, giving a sweet taste and flavour and resulting in higher consumer acceptability.

The effect of different methods of soymilk preparation on physio-chemical parameters such as solid yield, protein yield, solid contents, conductivity, total dissolve solid, pH, proximate composition (moisture, protein, fat, ash and carbohydrate), and hydroperoxide level was found to be significant at 1% level. Table 5 presents as a special case; ANOVA of hydroperoxide level and antioxidant activity of soymilk prepared by different methods. The statistical analysis indicates that these parameters of soymilk vary significantly with different methods of preparation. Also R^2 and coefficient of variation (CV) for each parameter indicate goodness-of-fit of the model, and CV of the variable under study was found to be very low (Table 5). The pairwise comparisons and minimum significant difference (MSD) at $P = 0.05$ level of significance for all the quality parameter were found to be different for different methods of soymilk preparation. It can be seen from Table 6 that the hydroperoxide level and antioxidant activity vary significantly for different methods of soymilk preparation according to minimum significance difference.

The overall comparative physico-chemical qualities of soymilk prepared by Illinois and deodorization methods were found optimum and acceptable. The hydroperoxide content, responsible for beany flavour was minimum in soymilk prepared by deodorization method followed by Illinois, rapid hydration hydrothermal, airless grinding, hot water grinding and traditional oriental methods. The shelf life of raw soymilk prepared by traditional oriental method and stored at 4°C was only one day, whereas that of raw soymilk prepared by other methods was 2–3 days. There was no change in taste and odour of pasteurised and refrigerated soymilk at 4°C up to seven days of storage. The organoleptic score of soymilk prepared by Illinois and deodorization methods was highest indicating good taste, flavour and acceptability among the people.

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Agronomic fortification of rice and wheat grains with zinc for nutritional security

Hari Ram[1,*], V. S. Sohu[1], Ismail Cakmak[2], Kuldeep Singh[1], G. S. Buttar[1], G. P. S. Sodhi[1], H. S. Gill[1], Indoo Bhagat[1], Parminder Singh[1], S. S. Dhaliwal[1] and G. S. Mavi[1]

[1]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141 004, India
[2]Sabanci University, Istanbul, Turkey

Zinc (Zn) deficiency is the most widespread micronutrient deficiency in crop plants and humans. Low intake of Zn through diet appears to be the major reason for the widespread prevalence of Zn deficiencies in human populations. Application of Zn fertilizer in soil having low Zn increased the grain yield in wheat up to 6.4–50.1%. However, soil Zn application increased the grain yield of rice only up to 7.2–14.8%. Soil having sufficient Zn had no or little effect on grain yield with soil Zn application. The application of foliar Zn with or without propiconazole resulted in significant increases in grain Zn irrespective of soil Zn status. Application of foliar Zn along with propiconazole at earing and milk stages proved beneficial in increasing grain Zn content in both rice and wheat. Hence agronomic biofortification is possible and could be considerably economical if used along with a fungicide depending upon appearance of a disease.

Keywords: Agronomic fortification, rice, wheat, zinc deficiency.

THE foodgrain production of the Indian subcontinent improved tremendously after the introduction of high-yielding, dwarf, fertilizer-responsive varieties of rice and wheat during 1966–68, progress in manufacture and consumption of chemical fertilizers, increase in irrigation facilities and development of rural infrastructure[1]. Rice and wheat constitute nearly two-thirds of the energy needs of humans in India[2]. Rice–wheat cropping system occupies about 10 million ha in the Indo-Gangetic Plains of India and is spread over the states of Punjab, Haryana, Uttar Pradesh, Bihar and West Bengal[1]. This cropping system has led to a significant decline in the area under legumes, which are a rich source of proteins and micronutrients. Till recently, the three major micronutrient deficiencies recognized globally were vitamin A deficiency leading to blindness, iron deficiency causing anaemia, and iodine deficiency responsible for goitre. Deficiency of zinc (Zn) has received global attention recently. Graham and Welch[3] reported that 50% of the soil used for cereal production in the world contains a low level of plant available Zn, which reduces not only grain yield but also nutritional quality. Zn deficiency in humans causes a wide range of health complications, including impairment in the immune system, learning ability and physical growth, and increase in mortality and infections[4]. Children are particularly more sensitive to Zn deficiency, which has been shown to be a major cause of death among children all over the world. It is responsible for nearly 450,000 deaths in children under the age of 5 years, which corresponds to 4.4% of the deaths among children less than 5 years of age globally[5].

Zn has been found useful in improving yield and yield components of wheat[6–8] and rice[9]. It is high time that along with food security due attention should also be given to adequate micronutrient nutrition. Soil and foliar Zn fertilization has shown good response in a number of crops including rice and wheat. Development of a new variety by conventional plant breeding is genetic biofortification, whereas agronomic biofortification is enrichment

*For correspondence. (e-mail: hr_saharan@yahoo.com)

of micronutrients in the grains by application of appropriate fertilizers. Fertilization approach is cheaper, faster and safer and can be applied to a number of crops. The application of micronutrients at appropriate stage with the right concentration is important, which plays a major role in obtaining promising results. The present study was therefore conducted under field conditions in different ecological zones of Punjab, India, to investigate the role of soil and foliar applied Zn fertilizers in combination with commonly used fungicide, propiconazole 25 EC, in improving cereal grain Zn.

Rice genotype PR 120 was grown at all the locations, except Pusa 1121 (basmati rice) used in Bhagatpur in 2009. This short-statured and high-yielding variety has been developed for specific environments and cropping systems; it is of short duration and consumes less water. The wheat varieties used were PBW 550 and DBW 17, developed by Punjab Agricultural University, Ludhiana and Directorate of Wheat Research, Karnal respectively. These varieties are also short-statured and have higher harvest index.

Field experiments were conducted during June 2009 to September 2011 in different ecologies of Punjab. The soils were normal in pH and EC with low available N, medium in P and sufficient in K. The soils at Gurdaspur and Bathinda were low in diethylene triamine pentacetic acid (DTPA) extractable Zn (<0.6 mg kg^{-1}), but not at other sites.

The region has a subtropical climate, with hot, wet summers for rice and cool, dry winters for wheat. Average annual rainfall is 734 mm, constituting 44% of pan evaporation. The depth to the groundwater is over 15 m.

The experiment was initiated with rice crop in 2009 in Punjab representing the Trans Indo-Gangetic Plains (IGP) of India. It comprised of two treatments, i.e. no Zn and soil Zn application (50 kg $ZnSO_4 \cdot 7H_2O$ ha^{-1}) in a randomized complete block design with four replicates. From wheat season (2009–10), additional treatments were added to the experiment, i.e. (i) no Zn, (ii) application of $ZnSO_4 \cdot 7H_2O$ in the soil (50 kg ha^{-1}) along with foliar Zn application at 0.5% of $ZnSO_4 \cdot 7H_2O$ (at earing and at milk stage), and (iii) treatment (ii) + application of Zn along with propiconazole fungicide (F). In 2010–11, one more treatment ($ZnSO_4 \cdot 7H_2O$ ha^{-1} @ 50 kg ha^{-1} in the soil) was added for both wheat and rice.

Plot size was 10 m × 2 m, with earth bunds around each plot to avoid movement of Zn fertilizer across the plots. After pre-irrigation to both rice and wheat, the field was ploughed with tractor-driven harrows and cultivators. Rice was seeded (20 kg ha^{-1}) on the beds for raising nursery. The nursery of 30 days age was transplanted manually in the experiment. Irrigation water was kept standing in rice for the first two weeks after transplanting and later irrigation was applied two days after evaporation of water in the field. Rice was transplanted during all the years in June, keeping row-to-row spacing of 20 cm and plant-to-plant of 15 cm. Zn fertilizer was applied before transplanting of rice crop. No P and K fertilizers were applied to rice crop, but N was applied @120–150 kg ha^{-1} at different locations. Where the soil was loamy sand (Ferozepur and Ludhiana), higher fertilizer dose (150 kg ha^{-1}) was used. To rice crop, one-third of N was applied at sowing, the remaining N was top-dressed in two equal split doses, i.e. 3 and 6 weeks after transplanting of the nursery. Weeds were well controlled in rice using Butachlor 50 EC within two days of transplanting of the crop as pre-emergence application. Rice crop was harvested during 15–22 October.

Wheat (cultivar PBW 550 2009–10/DBW 17 2010) was sown using 112.5/100 kg ha^{-1} seed rate with seed-cum-fertilizer drill at 20 cm of row spacing. Fertilizer dose of 150 kg ha^{-1} N was applied to wheat at Ludhiana and Bathinda, while 120 kg ha^{-1} of N was applied at other locations. Phosphorus @26 kg P ha^{-1} was applied at the time of sowing. To wheat, half of N and full doze of P were applied at sowing and the remaining N was top-dressed with first irrigation. Urea and diammonium phosphate (DAP) were used as the source of nutrients. In wheat, Arelon 75WP (isoproturon) at 1.25 kg ha^{-1} (35 DAS) followed by 2,4-D sodium salt (80%) at 0.625 kg ha^{-1} were used for controlling both grass and broadleaf weeds respectively. Both rice and wheat were harvested manually and the stubbles were removed from ground level. Wheat crop was harvested between 6 and 14 April during different years of the study period.

Each plot was harvested leaving non-experimental area for grain and straw yields. The rice crop was harvested and threshed manually, but wheat was harvested manually and threshed with machine.

The threshing was done with hands for determination of Zn in the grains. Grain was analysed with an atomic absorption spectrophotometer (model AAS FS 240 Varian, Australia) using standard procedures. Soil Zn and grain Zn were analysed in different laboratories to ensure precision.

The economic analysis was carried out using actual expenditure of activities/input and the prevailing rates for rice and wheat crops produced separately. The gross and net returns were calculated as follows.

Gross returns = Crop yield (t/ha) price of the produce (US$).

Net returns = Gross returns (US$) – cost of cultivation (US$).

The net returns were presented graphically across the locations.

The data collected were analysed using analysis of variance (ANOVA) by IRRISTAT v. 5.0 and Genstat v. 7.1. Treatment means was compared by DAP least significant difference (LSD) at $P = 0.05$.

Table 1. Effect of various Zn fertilizer treatments on grain yield (t ha^{-1}) of rice during 2009–11 at three locations in Punjab, India

	2009			2010				2011				
Location	No Zn	Soil Zn	LSD (*P* = 0.05)	No Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD (*P* = 0.05)	No Zn	Soil Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD (*P* = 0.05)
Patiala	5.12	5.47	0.25	5.22	5.82	5.92	NS	5.21	5.71	5.81	5.92	0.21
Ferozepur	4.08	4.30	0.10	4.32	4.93	4.89	NS	4.53	4.89	4.92	5.03	0.22
Bhagatpur	2.87	3.17	0.14	4.53	5.24	5.33	NS	5.02	5.66	5.83	5.91	0.41
Mean	4.02	4.31	0.17	4.72	5.33	5.42	NS	4.92	5.42	5.52	5.20	0.31
% Increase		7.21			12.92	14.83			10.16	12.20	5.69	

NS, Not significant.

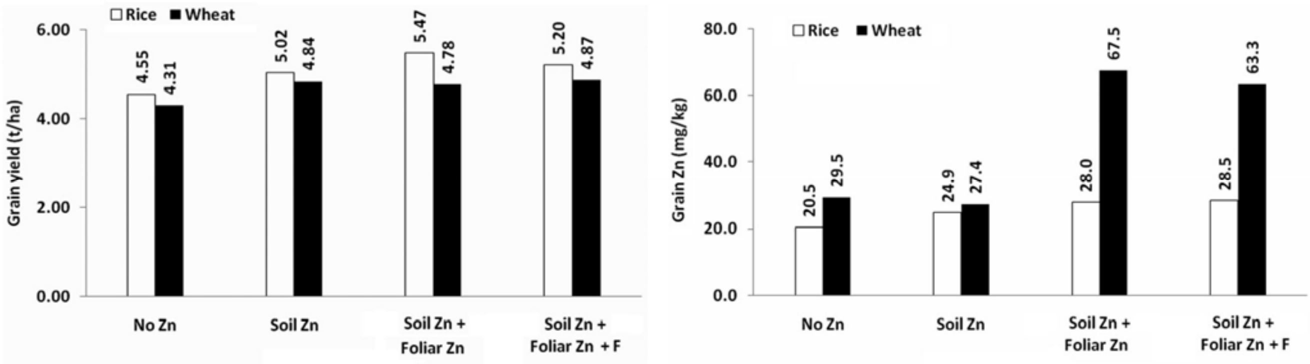

Figure 1. Effect of various zinc fertilizer treatments on the grain yields of rice and wheat across locations and years and grain zinc.

In 2009, rice grain yield obtained at Bhagatpur was less compared to other locations; this was due to basmati variety (Pusa 1121) sown at the location (Table 1). Application of soil Zn significantly improved grain yield at all the locations. The highest response in grain yield over no Zn was recorded at Bhagatpur (10.5%), followed by Patiala (6.8%) and Ferozepur (5.4%). In 2010, the results were non-significant but the trend was in favour of soil Zn application. In 2011, the highest grain yield was recorded in soil Zn + foliar Zn + propiconazole, which was statistically at par with soil Zn and soil + foliar Zn. On the basis of pooled mean grain yield over the locations, the increase was 7.2% in 2009, 12.9% in 2010 and 10.2% in 2011. On the basis of mean across the years and locations, soil Zn application, soil Zn + foliar Zn and soil Zn + foliar Zn + propiconazole increased the grain yield by 10.3%, 20.2% and 14.3% respectively. This may be due to response of rice crop to soil Zn application in marginal soils (Figure 1).

In 2009, the increase in rice grain Zn with soil Zn application over no Zn was in the order Ferozepur > Patiala > Bhagatpur (Table 2). Application of soil Zn resulted in lesser increase in the grain Zn compared to foliar Zn application. This may be due to less uptake of Zn from the soil by the rice roots. Soil application could not develop the Zn gradient in leaves to translocate Zn to the developing rice grains. In 2010, at Ferozepur and Bhagatpur the grain Zn content in soil Zn + foliar Zn + propiconazole was statistically on par with soil Zn + foliar Zn, but significantly higher than no Zn application. This may be due to non-appearance of diseases at these locations. During 2011, at Patiala and Ferozepur the highest grain Zn was recorded in soil Zn + foliar Zn, which was statistically on par with soil Zn + foliar Zn + propiconazole but significantly higher than no Zn and soil Zn treatments. Soil Zn also slightly increased the grain Zn, but it was not significantly different from no Zn treatment. However, at Bhagatpur the grain Zn was highest for soil Zn + foliar Zn + propiconazole treatment. This indicates the compatibility of foliar Zn + propiconzole in rice crop, which also saves the separate cost involved in the application of foliar Zn for grain Zn enrichment. On the basis of mean across all the locations both soil Zn + foliar Zn and soil + foliar Zn + propiconazole treatments recorded similar grain Zn, which was significantly higher than soil Zn and no Zn treatments. Foliar Zn applications near flowering stage may be one way to increase Zn in grains as the uppermost leaves were readily exposed to foliar sprays[9]. The spraying of 3.0 kg $ZnSO_4 \cdot 7H_2O$ in two applications at the flowering and early grain development stages increased the grain Zn significantly irrespective of the soil types. The results suggest that the negative effect of low DTPA extractable soil Zn status on grain Zn concentrations is not overcome

Table 2. Effect of various Zn fertilizer treatments on grain zinc (mg kg^{-1}) of rice during 2009–11 at three locations in Punjab, India

	2009			2010				2011				
Location	No Zn	Soil Zn	LSD (P = 0.05)	No Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD (P = 0.05)	No Zn	Soil Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD (P = 0.05)
Patiala	24.1	28.5	3.62	18.1	26.5	26.4	4.2	20.1	22.1	28.6	28.5	3.3
Ferozepur	18.3	22.1	2.90	22.2	29.5	29.6	5.1	21.2	23.1	27.9	27.8	3.9
Bhagatpur	21.5	24.4	2.39	18.2	25.9	26.1	5.3	20.6	21.9	29.1	29.2	3.7
Mean	21.3	25.0	3.0	19.5	27.3	27.4	4.9	20.6	22.4	28.5	28.5	3.6
% Increase		17.4			40.0	40.5			8.7	38.3	38.4	

Table 3. Effect of various Zn fertilizer treatments on grain yield (t ha^{-1}) of wheat during 2009–11 at five locations in Punjab, India

		2009–10				2010–11				
Location		No Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD (P = 0.05)	No Zn	Soil Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD (P = 0.05)
Zn-deficient	Bathinda	4.40	4.78	4.89	0.31	2.18	4.79	4.75	4.81	0.91
	Gurdaspur	4.54	4.73	4.95	0.15	4.23	4.83	4.83	5.03	0.16
	Mean	4.47	4.76	4.92	0.23	3.21	4.81	4.79	4.92	0.54
	% Increase		6.41	10.03			50.08	49.45	53.51	
Zn-sufficient	Ferozepur	4.13	4.23	4.33	NS	4.33	4.45	4.46	4.48	NS
	Patiala	4.80	5.10	4.91	NS	5.02	5.2	5.23	5.35	NS
	Bhagatpur	4.61	4.75	4.69	NS	4.86	4.95	4.93	5.23	0.13
	Mean	4.51	4.69	4.65	NS	4.74	4.87	4.87	5.02	0.04
	% Increase		3.97	2.92			2.74	2.89	5.98	
Overall mean		4.50	4.72	4.75	0.20	4.12	4.84	4.84	4.98	0.16
% Overall increase			4.94	5.75			17.46	17.36	20.76	

by application of Zn fertilizer at doses recommended for better yields of rice and wheat. Similar results were also reported elsewhere[10]. The best way of biofortification is through supplying the biofortified rice and wheat to reach rural populations in remote areas of India, such as IGP, who may not consume biofortified processed foods[11]. Farmers apply Zn fertilizer only if they expect a yield response as obtained in the present study. The foliar application of Zn in cereals at flowering or early grain development stages is uncommon in India, as there is no yield response. So when the farmers apply fungicides like propoiconazole for control of various diseases in rice, zinc sulphate heptahydrate can be mixed with the fungicide to get positive effect of fortification of Zn in cereal grains. Based on the overall mean across years and locations, soil foliar Zn application without or with fungicides increased the grain Zn content by 21.5–39.0% over no Zn application (Figure 1).

The response of wheat grain yield to soil Zn application was observed in the order Bathinda > Patiala > Gurdaspur > Bhagatpur > Ferozepur (Table 3). At Bathinda grain yield recorded in soil Zn + foliar Zn + propiconazole was statistically on par with soil Zn + foliar Zn, but significantly higher than no Zn application. However, at Gurdaspur the highest grain yield was recorded in soil Zn + foliar Zn + propiconazole, which was significantly higher than soil Zn + foliar Zn and no Zn. The grain yield recorded in soil Zn + foliar Zn was significantly higher than no Zn. Although wheat grain yields at Ferozepur, Patiala and Bhagatpur were similar, the trend was similar as recorded at Bathinda and Gurdaspur. During 2010–11, the grain yield recorded at Patiala and Ferozepur was non-significant. This is because yellow rust could not infect the experimental crop during that year of experimentation. At Bathinda, high soil pH and high levels of $CaCO_3$ and low levels of organic matter and soil moisture were predominantly responsible for low availability of Zn to plant roots. It has also been reported[6] that Zn application improved biological yield as well as grain yield of wheat grown on calcareous soils as shown in the present study as well. Higher grain yield of wheat was reported with the application of Zn in the soils[12,13]. At Gurdaspur the grain yield was highest in soil Zn + foliar Zn + propiconazole, which was significantly higher than the rest of the treatments. This may be because the fungicide was able to control the yellow rust disease in wheat. At Bhagatpur the grain yield recorded in soil Zn + foliar Zn + propiconazole was significantly higher than no Zn, soil Zn and soil Zn + foliar Zn. On the basis of pooled mean across soil-deficient Zn locations, the highest grain yield was recorded in soil Zn + foliar Zn + propiconazole, which was 10.0% higher in 2009–10 and 53.5% higher

Table 4. Effect of various Zn fertilizer treatments on grain zinc (mg kg^{-1}) of wheat during 2009–11 at five locations in Punjab, India

Location		2009–10				2010–11				
		No Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD ($P = 0.05$)	No Zn	Soil Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD ($P = 0.05$)
Zn-deficient	Bathinda	25.0	81.0	70.0	13.5	22.7	24.7	72.2	73.5	18.4
	Gurdaspur	34.3	69.8	61.3	4.8	25.3	27.2	60.4	60.3	13.9
	Mean	29.6	75.4	65.6	9.1	24.0	25.9	66.3	66.9	16.1
	% Increase		154.4	121.5			7.9	175.8	178.5	
Zn-sufficient	Ferozepur	48.7	63.0	65.3	NS	26.5	28.7	61.6	61.9	18.2
	Patiala	34.0	64.0	53.7	17.2	30.2	31.2	61.2	59.2	16.6
	Bhagatpur	25.8	77.0	64.8	12.2	22.4	25.3	64.2	63.2	13.0
	Mean	36.1	68.0	61.3	NS	26.3	28.4	62.4	61.4	15.9
	% Increase		88.2	69.5			7.9	136.7	133.2	
Overall mean		33.5	71.0	63.0	0.2	25.4	27.4	63.9	63.6	0.2
% Overall increase			111.6	87.9			7.9	151.5	150.3	

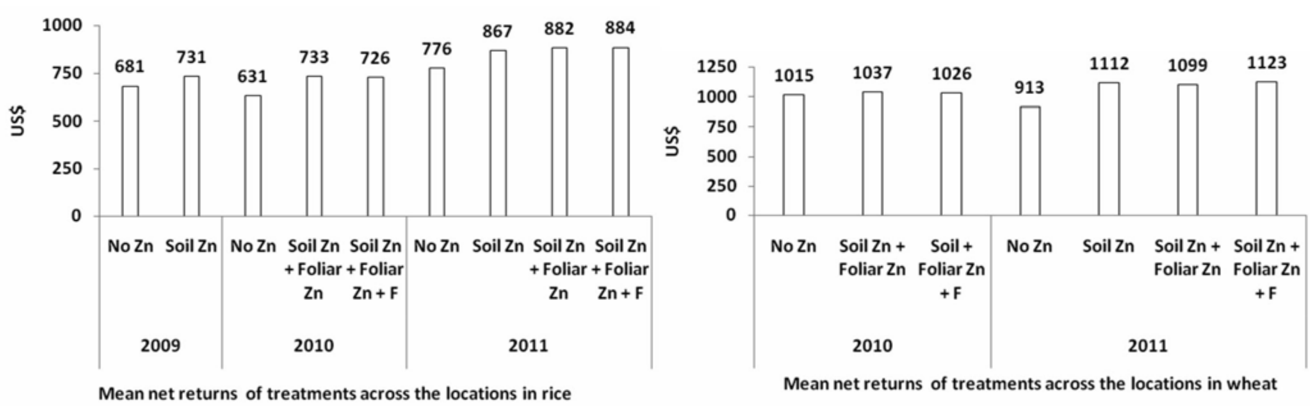

Figure 2. Effect of various zinc fertilizer treatments on net returns (US$) of rice and wheat.

than no Zn application. It was statistically similar to grain yield obtained in soil Zn + foliar Zn treatment. The highest grain yield on the pooled mean across the locations recorded in soil Zn + foliar Zn + propiconazole may be due to proper control of yellow rust disease. The positive effect of Zn applications on plant growth leading to increased leaf area index, plant height, number of fertile tillers m^{-2}, number of filled spikelets/spike, spike length, grains/spike, biological and straw yields and 1000-grain weight culminating in improved grain yield were also reported[14]. The percentage increase in grain yield in Zn-deficient soils was more than Zn-sufficient soils. This may be due to the effect of Zn fertilizer to make more available Zn to the crops, which ultimately increased the grain yield. Wheat grain yield across the years and locations increased from 10.9% to 13.0% in soil Zn + foliar Zn with or without fungicide (Figure 1).

During 2009–10, wheat grain Zn was significantly higher at all the locations except at Ferozepur (Table 4). The highest grain Zn was recorded in soil Zn + foliar Zn, which was statistically on par with soil Zn + foliar Zn + propiconazole, but significantly higher than no Zn application at all the locations, except Ferozepur, where the highest grain Zn was significantly higher than soil Zn + foliar Zn + propiconazole. On the basis of mean at Zn-sufficient locations, the highest grain Zn was recorded in soil Zn + foliar Zn, which was statistically on par with soil Zn + foliar Zn + propiconazole but significantly higher than no Zn application. During 2010–11, again the highest grain Zn was recorded in soil Zn + foliar Zn, except at Bathinda and Ferozepur, where the highest grain Zn was recorded in soil Zn + foliar Zn + propiconazole. At Bhagatpur the grain Zn was similar in both soil Zn + foliar Zn and soil Zn + foliar Zn + propiconazole treatments. At Gurdaspur and Patiala, the highest grain Zn was recorded in soil Zn + foliar Zn, which was statistically on par with soil Zn + foliar Zn, but significantly higher than no Zn treatment. On the basis of overall mean across the locations, the highest grain Zn was recorded in soil Zn + foliar Zn, which was statistically on par with soil Zn + foliar Zn + propiconazole. Under Zn-deficient conditions (Bathinda), Zn concentration in the grains was lower compared to the other locations having sufficient soil Zn. On the basis of overall mean the percentage increase in grain Zn in wheat with soil Zn + foliar Zn application alone or in combination with propiconazole was higher (121.5%–154.4% in 2009–10 and 175.8%–178.5% in 2010–11) in Zn-deficient soils compared to Zn-sufficient soils (69.5%–88.2% in 2009–10 and 133.2%–136.7% in 2010–11). The grain Zn in the Zn-deficient soil at Bathinda and Gurdaspur was low due to less available DTPA-extractable Zn. On the basis of overall mean, the highest grain Zn was recorded in soil Zn + foliar Zn, which was statistically on par with soil Zn + foliar Zn + propiconazole in 2010–11. This may be due to non-appearance of yellow rust at most of the locations. The grain Zn concentration was not influenced by fertilizer Zn application in the soil. This may be because translocation from soil to plant leaves is not so significant even at low DPTA-extractable sites. On the basis of mean across the locations and years, the foliar application of Zn with or without fungicide increased the grain Zn from 114.6% to 128.8% over no Zn application (Figure 1). Propiconazole is effective against yellow rust in wheat[15]. Higher order increase of grain Zn in deficient soil Zn locations may be due to enhanced effect of soil as well as foliar Zn applications[16,17].

In rice, during 2009, soil Zn application recorded the higher returns (Figure 2). During 2010, soil Zn + foliar Zn recorded the highest or similar returns as recorded in soil Zn + foliar Zn + propiconazole, but was higher than no Zn application. During 2011, soil Zn + foliar Zn + propiconazole recorded the highest returns across all locations. The higher returns in soil Zn or soil Zn + foliar Zn and soil Zn + foliar Zn + propiconazole may be due to higher grain yields recorded with the treatments.

Across the locations, during 2010, the net returns in wheat were highest with soil Zn + foliar Zn. Soil Zn + foliar Zn + propiconazole recorded the highest net returns in 2011. This was due to higher grain yield of wheat recorded in this treatment.

Thus soil Zn application increased wheat grain yield up to 50% and rice grain yield only up to 14.8%. Soils rich in Zn showed no or little effect on grain yield when Zn was applied. Application of foliar Zn with or without propiconazole resulted in significant increase in grain Zn, which was 38–40% in rice and 87–150% in wheat, over no Zn. Foliar Zn along with propiconazole fungicide at earing and milk stages proved most beneficial in increasing Zn content in the grains. This not only helped to increase grain Zn but was also most economical. However, fungicide application will depend upon the appearance of a disease and hence has value under specific conditions.

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