



ZINC ALLOCATION AND ITS RE-TRANSLOCATION IN WHEAT AT DIFFERENT GROWTH STAGES

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Abstract

The study provides an in-depth analysis and investigation of the allocation of zinc (Zn) and its re-translocation in different parts of the wheat plant at various growth stages. Four levels of Zn were applied in soil *viz.* 0, 5, 10 kg Zn ha⁻¹ and soil @5 kg Zn ha⁻¹ combined with two foliar applications @ 0.5% at tillering and pre-flowering stages. Foliar-applied zinc increased its concentration in all parts of the plant, however, the relative distribution of it to the vegetative structures and reproductive structure (spike) gradually increased from pre-flowering to panicle initiation stage due to significant proportion of Zn allocated in reproductive part. At maturity, foliar application of Zn significantly increased grain Zn concentrations. The allocation of grain Zn was almost double and more than double in comparison to straw and husk respectively. The yield was significantly increased by the application of zinc sulphate monohydrate along with two foliar sprays. Knowledge of Zn allocation and re-translocation in wheat is of paramount importance for improving fertilization strategies and contribute to human nutrition.

Key words: foliar application, remobilization, translocation, wheat, Zinc concentration.

Introduction

Zinc is an essential nutrient for the plant, human and animal, but it is unfortunately deficient in most of the soils because of intensive cropping system, imbalance use of fertilizer application, cultivation of high yielding variety crop and ignorance of micronutrient fertilizers (Alloway, 2008, Shukla *et al.* 2014), so its deficiency is reported in the plant, animal and human diet (Cakmak 2006). Zinc deficiency is a well-documented health issue globally and almost one-third of the world's population is affected by Zn deficiency (Alloway, 2009). In India, all major nutrients (nitrogen, phosphorus and potassium) are deficient but among micronutrient zinc deficiency is the highest (Shukla *et al.* 2014). Zinc supplementation helps linear growth, reduces child mortality as well as reduces the severity and duration of diarrhoea and respiratory infections. The daily intake of Zn as a diet for an infant, children less than 10 years, female more than 10 years,

and women during pregnancy are 5 mg/day, 10 mg/day, 12 mg/day and 15 mg/day respectively as recommended by the studies of Welch (2001).

Researchers have emphasized the importance of micronutrients in maintaining adequate growth and development of crops and thereby good human health (Shukla *et al.* 2014; Joy *et al.* 2015, Cakmak, 2016). Zinc is reported to be involved in many enzymes as a cofactor and helps in activation of several enzymes like dehydrogenase, aldolase, isomerase, transphosphorylase, and DNA polymerase. Zinc influences the synthesis of some growth hormones in plants and is involved in auxin metabolism like tryptophan synthase (Marschner, 1995).

Zinc is also involved in a large number of fundamental functions in plants like synthesis of tryptophan (a compound needed for production of auxins), cell membrane integrity, cytochrome and chlorophyll synthesis, component of carbonic anhydrase which facilitates the

transfer of carbon dioxide for photosynthetic CO₂ fixation, and superoxide dismutase (SOD) protects membrane lipid and proteins against oxidation (Rattan, 2015) thus physiological processes like photosynthesis, water transport, opening and closing of stomata, etc. are adversely affected due to its deficiency (Marschner 1995; Welch 1999). The symptoms of Zn deficiency appear usually on the lower leaves, particularly towards the base. While in case of wheat, fading of the middle lamina on the older leaves are the Zn deficiency symptoms. Severe Zn deficiency causes a delay in maturity and grain formation and ultimately the yield is reduced (Cakmak 2008, Alloway 2009, Hussain *et al.* 2012). Longnecker and Robson (1993) reported that Zn can mobilize considerably within the plant with the degree of mobilization depending on the Zn supply. The total Zn concentration is not a dependable key to show the ability of the soil to supply available Zn for plants. Availability of Zn in soil depends on pH, lime content, organic matter content, amount and type of clay minerals and amount of applied P fertilizer (Havlin *et al.* 2007). Soil with intensive cropping system is more likely to have Zn deficiency. At the time of green revolution, high yielding varieties along with major nutrient fertilizer were used which resulted in decrease in uptake of Zn by plants from soil and its mobility within plants also reduced (Graham *et al.*, 2007).

The severity of zinc deficiency in soils is determined by diethylene triamine penta-acetic acid (DTPA)-extractable Zn (Shukla *et al.* 2014). Zinc deficiency correction by soil applications of Zn fertilizers is a common practice, but only 20% of the fertilized Zn is accessible for plant uptake and remaining 80% is adsorbed on soil colloid (Singh, 2005). Therefore, micronutrients application to soil may not fulfil crop requirement. To overcome micronutrients deficiency, the foliar application would be a good alternative because it seem like more helpful as compare to soil application for efficient use of nutrients and strategy to improve zinc concentration in edible parts (Cakmak 2008). In past few years, an extensive progress has been reported on the influence of the foliar application of Zinc in wheat grain because of its low application rates and less Zn losses through soil fixation. Foliar Zn may be taken up by leaf epidermis which retranslocate and gets relocated into the grain through the phloem (Haslett *et al.*, 2001). Zou *et al.*, (2012) reported that Zn fertilizers may increase grain Zn content in wheat depending on the extent of Zn deficiency in soil.

Wheat is the most commonly grown crop worldwide. It is one of the most important crops for human consumption. It contributes to about more than 50% of the daily calorie intake in most of the Asian countries and

it is the second important staple food crop after legumes. The Zn deficiency is most widely occurring in cereal cultivated soils due to the use of NPK fertilizers over many years for getting high crop yield as a result crop extracted more micronutrient from the soil which limits the bioavailability of micronutrient like zinc (Cakmak 2009; Monreal *et al.* 2015). The reduction in average yield of wheat is also considerable due to Zn deficiency. Therefore, it is necessary to improve Zn nutritional status in crops as well as in Soil. The wheat is cultivated commonly in areas having Zn deficiency (Alloway, 2009).

The current field study investigated how levels and method of zinc application affected zinc concentration, translocation and reallocation by wheat.

Material and Methods

Experimental Site

Field plot experiments were conducted from mid-December to the following mid-April for two cropping seasons (2012– 2014) at experimental area of the Department of Soil Science and Agricultural Chemistry, Birsa Agriculture University, India at 23°172 N, 85°192 E and 625 msl. The experimental site was having mean annual temperature of 29°C and a mean annual precipitation of 1398 mm. About 80% of the precipitation falls during the rainy season (June to September).

Initial Soil Test Values

The soil at this location was acidic with sandy loam texture. Particle-size distribution was determined by hydrometer method (Black, 1965), soil pH by Glass electrode pH meter (Jackson, 1973) and EC by Systronics Electrical conductivity meter (Jackson, 1973) and organic carbon by the Walkley and Black's rapid titration method (Jackson, 1973). Soil available N by alkaline KMnO₄ method (Subbiah and Asija, 1956), available P by Bray P1 method (Bray and Kurtz, 1945) and available K was determined by Neutral 1 N NH₄OAc extraction method (Hanway and Hiedal, 1952). Available Fe, Zn, Mn and Cu in the soil were first extracted by DTPA and then were read by atomic absorption spectrophotometer (AAS) (Lindsay and Norvell, 1978). This soil pH (4.68), O.C.% (0.41), available N (330 kg ha⁻¹) available (P 30.6 kg ha⁻¹) and (142.5 kg ha⁻¹) available K, DTPA extractable Zn, Fe, Mn and Cu content were 1.08, 15.27, 6.91, 1.02, mg kg⁻¹, respectively.

Treatments and experimental Design

Wheat (*Triticum aestivum* L.) var. K9107 was selected for the experiment that continued for two cropping seasons. The layout of the experiment was a randomized complete block design with three replicates.

The treatments consisted of four levels of applied Zn: 0 (T_1), 5(T_2), 10(T_3) kg Zn ha⁻¹ and 5(T_4) kg Zn ha⁻¹ along with two foliar sprays of ZnSO₄·H₂O (0.5%) at tillering and before flowering stage in both the wheat cropping seasons.

Cultural Operations

Before sowing, applications of fertilizers included half of nitrogen, total phosphorus and total potassium applied as basal dose in the form of DAP, urea and MOP (120: 60: 40) at the time of sowing and one fourth of nitrogen was top-dressed after 30 DAS and rest one fourth after 60 DAS in the form of urea in splitted doses. Zinc was applied as a soil application in the form of zinc sulphate monohydrate. Two foliar sprays in selected treatment in the form of zinc sulphate monohydrate (0.5%) were applied at the tillering and before flowering stage of wheat. To avoid contamination, a 50-cm-wide border was maintained between the Zn treated plots. Foliar Zn was applied with a manual, high-pressure sprayer without wind (<0.2 ms⁻¹).

Data Collection

At maturity, wheat was harvested from net plot area of each plot to determine the grain and straw yield. Samples were collected and separated into grain and straw. A harvest index was calculated as the percentage of grain yield relative to the grain plus straw yield.

Plant samples were collected at tillering, pre-flowering, panicle initiation and at maturity from each plot. From each treatment, five plants were selected randomly. Plant samples were washed quickly by tap water and then deionized water, and these samples were first sun-dried and then air-dried. The air-dried samples were divided into different parts *i.e.* tillering stage-whole plant; before flowering- upper leaf, lower leaf and stem; panicle initiation- stem, lower leaf, middle leaf, upper leaf and spike and after harvesting – straw, husk and grain for the study of zinc allocation and re-translocation in wheat.

Chemical Analysis

All samples were dried in an oven at 60°C for 72

hours and ground with a stainless steel grinder for Zn analysis. Half a gram (0.5g) of 100 mm mesh powdered plant sample was digested with diacid (HNO₃:HClO₄ in 10:4) mixture in a digestion chamber. After complete digestion, it was filtered (Whatman No. 42) in 25 ml volumetric flask and volume was made by thoroughly washing with double distilled water. This sample was preserved for Zinc estimation by using atomic absorption spectrophotometer.

Statistical Analysis

Fisher's method of analysis of variance was followed for analysis and the interpretation of the data was done as suggested by Panse and Sukhatme (1967). The level of significance used in 'F' test and 't' test was P=0.05. Critical difference was calculated whenever 'F' test was significant.

Results and Discussion :

Wheat Grain and Straw Yield

The difference in yield to Zn application was noticed in the field study during the study period. The growth response was significantly increased by 17% in grain yield and 11% in straw yield as compare to control (T_1) by the application of zinc sulphate monohydrate along with two foliar sprays *i.e.* T_4 (Table 1, Fig. 1). The pooled data revealed significantly superior grain yield under the levels of zinc application T_4 over the T_1 , T_2 , and T_3 . The response of the crop to different doses of zinc application, in terms of grain yield, seemed to be positive. Increases of yield were consistent for both grain and straw although the increases were significantly greater for grain yield than for straw yield. However, harvest index was significantly not affected by Zn application during both the years.

The maximum increase in grain, as well as straw yield, was observed when the Zn was applied @ 5kg Zn along with two foliar sprays (T_4). Wu *et al.*, (2010) and Boorboori *et al.*, (2012) also found an increase in grain yield of wheat crop by Zn application. These results

Table 1: Crop response to different levels of Zn application in wheat in terms of yield(q ha⁻¹) and harvest index (%)

Level of Zn application	2012			2013			Pooled		
	Grain yield	Straw yield	Harvest index	Grain yield	Straw yield	Harvest index	Grain yield	Straw yield	Harvest index
T_1	3.43	6.36	33.6	3.9	6.31	34.3	3.36	6.34	34
T_2	3.53	6.81	33	3.4	6.75	33.5	3.47	6.78	33.2
T_3	3.69	7.02	34	3.68	6.95	34.4	3.68	6.98	34.2
T_4	3.91	7.04	34.6	3.94	6.98	36.1	3.93	7.01	35.4
CD at 5 %	3.2	4.5	NS	3.9	4.7	NS	2.2	3.3	NS
CV %	10.4	8	6.8	12.9	8.4	11.5	7.3	5.8	4.7

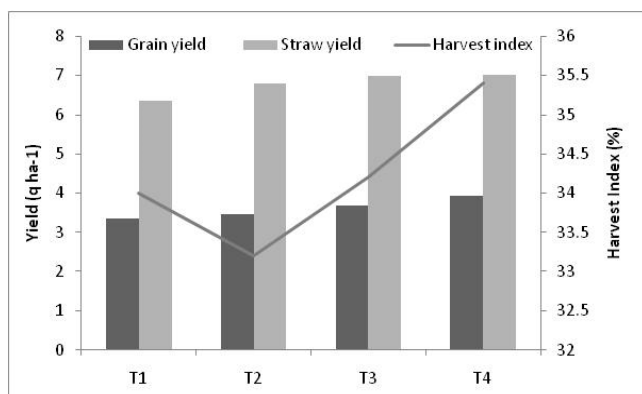


Fig. 1: Crop response to different levels of Zn application in wheat.

implied that wheat requirement of Zn was met from the soil along with two foliar sprays to attain full yield potential compared to soil application alone. The response of the crop to different levels of zinc application, in terms of grain yield, seems to be positive. The increase in yield of grain is attributable to enhanced physiology of plants with zinc application consequently correcting the efficiency of chlorophyll content, different enzymes, IAA hormone and improvement in nitrate conversion to ammonia in plant leading to better yield. (Hacisalihoglu *et al.*, 2003; Abbas *et al.*, 2010).

Allocation of Zn in plant parts at different growth stages

Table 2: Zn concentration (mg kg⁻¹) in different plant parts at tillering and pre-flowering stages in wheat.

Level of Zn application	Tillering stage			Pre Flowering stage								
	Tiller			Upper Leaf			Lower Leaf			Stem		
	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled
T ₁	42.6	42.5	42.5	36.5	42.5	36.1	47.5	35.6	47	36.8	46.4	37.3
T ₂	53.5	52.6	53.1	39	52.6	38.6	52.3	38.2	51.8	42.4	51.3	42.8
T ₃	55.4	55	55.2	45.7	55	45	51.7	44.4	51.1	44.5	50.5	44.3
T ₄	59.5	58.8	59.2	49	58.8	48.4	54.5	47.9	53.9	44.1	53.3	44.1
CD at 5%	3.6	6	3.6	4.8	6	3.5	4.6	3.2	3.8	NS	5.03	3
CV%	8.1	13.8	8.2	13.5	13.8	9.9	10.8	9.2	9	16.4	11.9	8.5

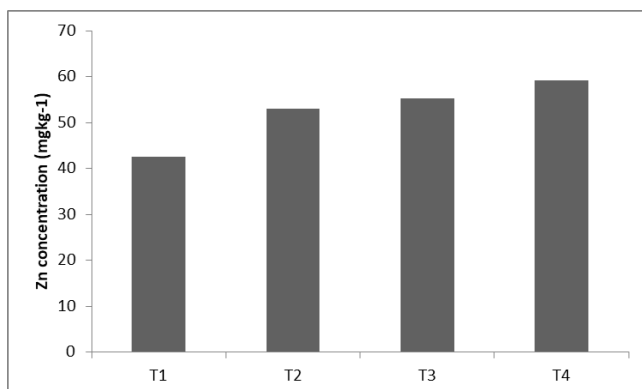


Fig. 2: Allocation of Zn at tillering stages

Zinc content in the different plant parts increased during the vegetative growth stage afterward remained comparatively stable during the reproductive stage. In general, the reproductive plant parts had the highest Zn concentration. However, concentrations decreased more promptly in stems than leaves. The pattern of Zn distribution differed among plant parts. The allocation of Zn to the vegetative organs reduced along with increased proportions in the reproductive structure.

Allocation of Zn at tillering stage

The Zn concentrations in tillers increased significantly with an increase in the dose of applied Zn and it was highest in case of T₄ (59.2 mg kg⁻¹) *i.e.* 5 kg Zn ha⁻¹ along with two foliar sprays (Table 2, Fig. 2).

Allocation of Zn at pre-flowering stage

The concentrations of Zn in the different plant parts increased during the pre-flowering stage (Table 2, Fig. 3). Overall, the treatment T₄ recorded the highest Zn levels in different plant parts. However, Zn content increased more rapidly in lower leaf (53.9 mg kg⁻¹) than stem (44.1 mg kg⁻¹) and upper leaf (48.4 mg kg⁻¹). Zinc accumulation in stem was about 30% whereas about 37% and 33% of Zn was accumulated in lower leaf and upper leaf respectively during pre-flowering stage. For the plant parts, the upper leaf followed by the stem had the

minimum Zn levels in the pre-flowering stage. The patterns of Zn accumulation in plant parts were similar in all levels of Zn application. Page and Feller (2005) reported that allocation of Zn depended on the age of plant and Zn content of source organs.

Allocation of Zn at panicle initiation stage

At panicle initiation stage the Zn content in the different plant parts decreased until a maximum was attained in spike (Table 3, Fig. 4). Even if the way patterns of Zn allocation were parallel in leaves and stems, stem had a lower Zn content which decreased more as compared to leaf. The Zn accumulation in spike (53.6

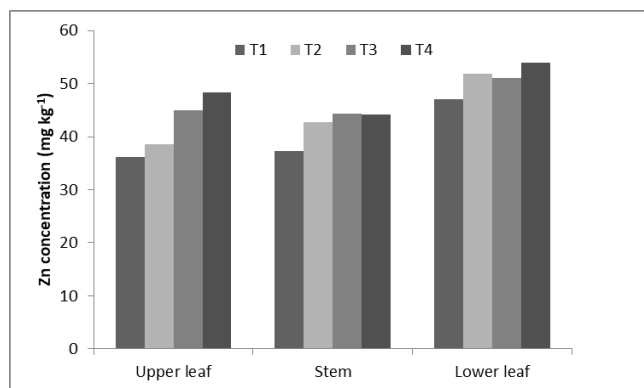


Fig. 3: Allocation of Zn at pre flowering stages

mg kg⁻¹), stem (22.7 mg kg⁻¹), lower leaf (41 mg kg⁻¹), upper leaf (32.8 mg kg⁻¹) and middle leaf (36.2 mg kg⁻¹). About 29% Zn was accumulated in spike whereas about 22%, 19%, 18% and 8% of Zn was accumulated in lower leaf, middle leaf, upper leaf and in stem respectively during panicle initiation stage. Overall, vegetative Zn allocation declined in reproductive stage with an equivalent increase in allocation to the spike. The proportion of phloem-mobile micronutrient (Zn) distributed to the stems and leaves declined at pre-flowering to panicle initiation stage. Lima *et al.*, 2015 reported that concentration of Zn was highest in the stem at vegetative branches and lowest in leaves in flowering branches.

The comparative distribution of Zn to the vegetative parts (*i.e.*, stem plus leaf) and reproductive structure (*i.e.*, spike) gradually increased from pre-flowering stage to panicle initiation stage as a result of a larger proportion of Zn distributed in reproductive part. Vegetative parts (*i.e.*, stem plus leaf) had comparatively small proportion of the plant Zn. Thus, plant parts in particular stems made up of the primary temporary sink for Zn retranslocated later into the other growing parts. Reallocation of Zn from the leaf canopy during the tillering and pre-flowering stages of the growing season was not readily noticeable. The magnitude of the remobilization of Zn was found in following order: stem < upper leaf < middle leaf < lower leaf < spike. The mobility of Zn in phloem is low and it

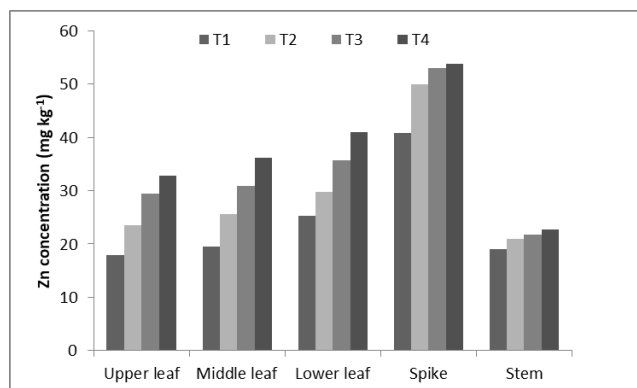


Fig. 4: Allocation of Zn at panicle initiation stages

decreases in leaf tissue as leaf ages. During the reproductive phase, a large amount of Zn is translocated from leaves to the reproductive parts (Lima *et al.*, 2014 and Pearson and Rengel, 1994). Reserved Zn (>70%) in the vegetative organs of wheat plants were re-translocated to other plant parts (Grusak *et al.*, 1999). The occurrence of higher Zn concentrations in lower leaves in comparison to upper leaves is the confirmation of Zn immobility while higher Zn concentrations in upper leaves is an indication of Zn mobility. Zn concentrations in the reproductive structure that is equal or exceeding leaf Zn concentrations also indicate phloem Zn mobility. Zn can be easily transported in the phloem *i.e.*, from old leaves to young tissues, indicating that phloem mobility of Zn (Haslett *et al.*, 2001, Erenoglu *et al.*, 2002).

Allocation of Zn at maturity

The accumulation of Zn in the developing grains (52.6 mg kg⁻¹) was higher than husk (13.3 mg kg⁻¹) and straw (25.1 mg kg⁻¹) at maturity (Table 4, Fig. 5). The addition of grain Zn was mostly redistributed from other plant parts. Foliar spray of Zn remarkable increases grain Zn concentrations. The allocation of grain Zn was almost double and more than double in comparison to straw and husk respectively.

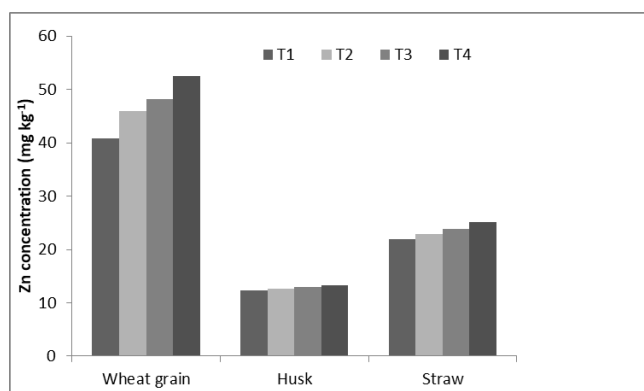
Zn can be easily transported in the phloem *i.e.*, from old leaves to young tissues, indicating that phloem mobility

Table 3: Zn concentration (mg kg⁻¹) in different plant parts at panicle initiation stage in wheat

Level of Zn application	Panicle Initiation stage														
	Upper Leaf			Middle Leaf			Lower Leaf			Panicle			Stem		
	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled
T ₁	18	17.9	17.9	19.5	19.5	19.5	25.2	25.5	25.3	40.6	41	40.8	19.2	18.8	19
T ₂	23.5	23.5	23.5	25.6	25.6	25.6	29.8	29.7	29.7	50.1	50	50	21.5	20.5	21
T ₃	29.4	29.5	29.5	31	30.8	30.9	35.7	35.6	35.7	52.8	53.1	53	22.2	21.3	21.7
T ₄	32.5	33.1	32.8	36	36.3	36.2	41.2	40.7	41	53.7	53.9	53.8	23	22.4	22.7
CD at 5 %	2.6	2.5	2.2	2.7	2	2	3	3.1	2.1	3.3	4	2.9	2.5	2	1.6
CV %	11.9	11.3	10	11.3	8.6	8.6	10.6	11.4	7.6	8.1	9.6	7.1	13.8	11.6	9.3

Table 4: Zn concentration (mg kg⁻¹) in different plant parts at maturity in wheat

Level of Zn application	Maturity stage								
	Wheat Grain			Husk			Straw		
	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled
T ₁	40.5	41.3	40.9	12.3	12.5	12.4	22	21.8	21.9
T ₂	45.5	46.5	46	12.6	12.6	12.6	22.9	22.9	22.9
T ₃	47.7	48.8	48.2	13.1	13	13	23.8	23.9	23.8
T ₄	52.2	53	52.6	13.2	13.5	1	24.9	25.2	25.1
CD at 5 %	2.4	2	1.5	NS	NS	NS	NS	1.9	1.5
CV%	6.2	5.1	3.7	11.7	10.4	7.4	13.4	9.5	7.7

**Fig. 5:** Allocation of Zn at maturity

of Zn (Haslett *et al.*, 2001, Erenoglu *et al.*, 2002). Although increasing additions of Zn to soil and soil along with the foliar application of zinc sulphate monohydrate significantly increased the Zn content in plant parts as compared to control, the effect of foliar application of zinc on the enrichment of Zn content was more prominent than soil application. By optimizing the timing of foliar spray of Zn, Zn concentration in grain could be further increased (Cakmak *et al.*, 2010b; Zhang *et al.*, 2010) and the soil along with foliar spray of Zn is the most efficient method for increasing Zn content in grain (Cakmak *et al.*, 2010a). Zinc fertilization is helpful for increasing yield as well as zinc concentration in grains (Torun *et al.*, 2001). Zn application in soil are less effective as compared to foliar application in wheat, it result in notable increases Zn content in grain in wheat (Cakmak *et al.*, 2010a,b).in wheat, about 28%, 15% and 58% Zn was accumulated in straw, husk and grain respectively.

Zinc applied to roots along with two foliar applications increased 28% Zn content in shoot and 58% in grain. Variations in Zn content in plant parts during growth were indicative of a reallocation of Zn in different plant parts. Loss of Zn from the stems was due to reallocation because it does not shed.

Conclusions

Our results indicate that the accumulation patterns were related to the Zn translocation and the mobility of Zn found to be more towards upper plant parts at the reproductive stage and highest toward grain at maturity stage of wheat. Soil along with foliar application of Zn signifies a very efficient and fast method for Zn enhancement of wheat grains. Such data can be used to implementing the fertilizer recommendation programs. High zinc content in grain is advantageous for human nutrition as well as for minimizing the health issues related to Zn deficiency.

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