Optimization of Zinc Application Rates With and Without Arbuscular Mycorrhizal Fungi for the Improvement in Maize Growth, Gas Exchange Attributes and Yield in Zinc Deficient Soil

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Abstract

Zinc (Zn) concentration in soil varies from deficient to toxic. Its deficiency, as well as toxicity through imbalance application, can reduce maize growth and yield. Therefore, balanced application of Zn is a necessity of time to save resources and to achieve optimum growth and yield in maize. Arbuscular mycorrhiza fungi (AMF) can provide tolerance to host plant against Zn induced stress. Inoculation of AMF not only helps in balance uptake of Zn but also enhance growth and yield of crops. That’s why in the current efficacious function of AMF, i.e., Glomus specie was asssed. Different application rates of Zn (0, 20, 40, 60, 80, 100 and 120 mg Zn/kg) were applied with AMF (AM) and without AMF (NM). Results showed that root colonization level was 45% higher in AMF inoculated plants as compared with non-inoculated plants. A significant increase in plant height (15%), number of leaves (35.4%), cob weight (4.39%), 1000 grains weight (10.5%) and biological yield (42.2%) signified the efficacious functioning of Zn20+AM over sole inoculation of AM. We also observed that AMF inoculation with Zn20 helped in improved photosynthesis, transpiration and stomatal conductance. Furthermore, both Zn20+AM and Zn20+AM were significantly better for the improvement in total soluble protein over AM in maize. Higher application rates of zinc, i.e., Zn80 and Zn120 induced Zn toxicity with (AM) and without (NM) AMF. In conclusion, Zn20+AM is an effective amendment to achieve better growth and yield of maize without Zn deficiency or toxicity.

1. Introduction

Maize (Zea mays L.) is the third most cultivated crop which is member of Poaceae (Gramineae) family. It has two growing seasons, i.e. spring and autumn [1]. Due to its two growing season and wide adaptability, it is cultivated worldwide. In Pakistan, it is cultivated in all four provinces among which Punjab and KPK are major maize producers and contributed about 97% of total production [2]. According to a survey, maize is cultivated on 1318,000 ha in Pakistan, providing 6.9000 tons of biological yield. Also, maize is considered as a nutrient exhaustive crop as it uptakes a high amount of nutrients from the soil. Unfortunately, our soils are unable to supply enough quantity of nutrients, especially zinc (Zn) which fulfil plant demands and improve crop yield [3–5]. Maize is highly sensitive to Zn deficiency that caused 40% yield reduction in maize yield [6, 7].

Zinc is considered as most required micronutrient among the micronutrients, for normal and healthy plant growth [3, 8–10]. It is a structural component or cofactor of various enzymes involved in many biochemical processes. In plants, it is involved in photosynthesis, carbohydrate metabolism, protein metabolism, pollen formation, auxin metabolism, maintain membrane integrity and induce tolerance against various stresses [11]. Total concentration of Zn in soil ranges from 10–300 mg Zn /kg soil, and the average is 50–55 mg Zn/kg soil. However, most of Zn form complexes with soil colloids and organic matter (OM) rendering bioavailability of Zn for plants. Therefore, a very minute concentration of Zn is available for plant uptake.
Plants uptake Zn in the form of \( \text{Zn}^{+2} \) and \( \text{ZnOH}^- \) from soil solution. Zn deficiency in soil is a global problem. Arid and semi-arid soils are usually deficient in Zn due to high fixation rate of Zn. Furthermore, high soil pH drastically reduced the soil Zn bioavailability. Soils of Pakistan mostly exhibit high soil pH, high \( \text{CaCO}_3 \) and low OM contents, thereby Zn deficiency is the major issue in most of the regions [12]. High input of phosphatic fertilizers also reduced Zn bioavailability due to the formation of insoluble Zn phosphates in soil. Soil microbial application technique is considered as the best among various available methods that are used to achieve plant Zn demand [3, 5, 13–15]. Commonly used soil microbial technique includes the application of plant growth-promoting bacteria, Zn-solubilizing bacteria and arbuscular mycorrhizal fungi. Arbuscular mycorrhizal fungi colonize the plant roots and form a symbiotic relationship with plants by providing/regulating nutrients, water and protection from pathogens. More than 90% of terrestrial plants are found to get benefited from this symbiosis (Smith and Read, 2008). Their contribution in regulating the immobile nutrient (P, Zn, Fe, Cu and K) from soil system to plants, is well known [15]. AMF also have the potential to mediate the toxic effects of metals (Zn) and imparted a protective effect to promote plants growth under metal-polluted conditions also [16]. It has been observed that AMF inoculated plants produced more biomass as compared to non-inoculated plants. Increased biomass is directly related to improved plant nutrition due to increased root colonization rate [17].

AMF contribution in Zn uptake was quantified by Jansa et al. [18] after that, Watts-Williams et al. [19] also quantified the total Zn uptake and mycorrhizal contribution and plant dependency on \( \text{R. irregularis} \). In wheat and barley, \( \text{R. irregularis} \) contributed up to 24.3% and 12.7% in Zn uptake, respectively [15]. Therefore, the current study was conducted to explore the potential benefits of AMF on growth and yield attributes of maize under different application rates of Zn. It is hypothesized that AMF can be helpful in decreasing the application rate of Zn. In addition, AMF can also play an imperative role in the improvement of growth, gas exchange attributes and yield of maize.

2. Materials And Methods

2.1. Experimental soil and location

A CRD pot experiment was conducted in the research area of Department of Soil Science, Bahauddin Zakariya University Multan, Pakistan. Zinc deficient (0.52 mg Zn/kg) soil was collected from Chak 5 Faiz location latitude 29.9 °N and longitude 71.5 °E.

2.2. Experimental soil characterization

Air dried soil of 2 mm mesh size was mixed sand at 8:2 ratio, termed soil. After mixing soil was analyzed for various physico-chemical properties. It contained 39% saturation, 54% water holding capacity, 0.14 dSm\(^{-1}\) ECe [20], 8.82 pHS [21], 6.5 mgkg\(^{-1}\) available P [22], 126 mg kg\(^{-1}\) extractable K [23], 0.43% organic matter [24] and 0.0215% organic N [25].

2.3. Pots preparation by addition of Zn and AMF
Non-draining pots were filled with 20 kg soil that inoculated with 5 g mycorrhizal inoculum Clonex® having 158 propagulegram⁻¹. *Glomus specie* was the major constituent of inoculum. In AMF controlled pots (M), Topsin M (Thiophanate Methyl 70% WP) was applied at 50 mg kg⁻¹ soil, for rendering AMF root colonization. Six levels of Zn concentrations 20, 40, 60, 80, 100 and 120 mg Zn kg⁻¹ soil as ZnSO₄ form, were applied along with control treatment. Treatments were referred to as Zn0, Zn20, Zn60, Zn80, Zn100 and Zn120.

### 2.4. Moisture maintenance and fertilizer addition

The moisture content in each pot was maintained at 60% of total WHC on weight basis [26] and the recommended doses of N, P and K fertilizers were applied at the rate of 200:150:100 kg ha⁻¹ [27].

### 2.5. Seeds sowing and thinning

Maize variety YH 1898 was used as a test crop in this study. Two weeks after germination, plants were thinned to two plants per pot. These two plants were irrigated until the maturity.

### 2.6. Morphological attributes

Plant agronomic parameters such as number of leaves were counter from counted from maize plants, plant height (cm) and stem girth (cm) was measured by measuring tape before harvesting in three replications.

### 2.7. Yield parameters

After harvesting yield traits such as cob weight (g) was calculated by weight balance, whereas cob length (cm) was measured by measuring tape in three replicates were recorded. Biological yield was noted at the time of maturity (115 days after sowing).

### 2.8. Gas exchange attributes

Stomatal conductance, photosynthetic rate and transpiration rate were measured at the tasselling stage by constant light intensity of photosynthesis device (1500 µmol m⁻² sec⁻¹), CO₂ amount (400 µmol) and airflow (500 µmol s⁻¹) and when photosynthesis gained steady state, the measurement was recorded by LCi- SD Ultra Compact Photosynthesis System® [28–30].

### 2.9. Total soluble protein

Leaf stored at -80 °C in freezer for preparation of enzyme extracted in potassium phosphate buffer (pH 4), vortexed and centrifuged. Bradford reagent was added, and absorbance was measured by using ELISA plate at a wavelength of 595 nm [31].

### 2.10. AMF colonization determination/measurement

Roots were harvested for AMF root colonization assessment by following gridline intersect method [32]. Briefly, roots were washed in 10% KOH solution and tryphan blue stain was used for staining to observe
arbuscular mycorrhizal colonization in root tissues of maize by following method developed by Phillips and Hayman [33].

2.11. Statistical Analysis

Data were statistically analyzed by using two way- Analysis of variance (ANOVA) [34]. Difference of treatments means were analyzed by least significance difference (LSD) test using 5% level of significance using Statistical Package Statistix 8.1 (Tallahassee Florida, USA) and Origin 2020b. Pearson correlation and principal components analysis was conducted by using Origin 2020b.

3. Results

3.1. Root colonization

Inoculation of AMF significantly (p ≤ 0.05) increased the colonization percentage irrespective of soil Zn level up to 60 mg Zn kg\(^{-1}\) application, however higher application resulted in decreased colonization percentage. Non-inoculated (NM) maize roots showed up to 45% root AMF colonization level, as shown in Fig. 1. Whereas, AMF inoculated (AM) maize plant roots were showed higher colonization with AMF (Fig. 1). Colonization percentage was significantly (p ≤ 0.05) high, with an average of 45% in AMF inoculated plants irrespective of AMF inoculation, Zn application reduced the AMF colonization rate after 20 mg Zn kg\(^{-1}\) application. More drastic effect of Zn application was observed in NM plants. Moreover, zinc was negatively correlated with AMF root colonization as Zn application rate increased.

3.2. Plant Height

Application of different levels of Zn with (AM) and without AMF (NM) significantly affected the plant height of maize. Treatment Zn20 + AM and Zn40 + AM differed significantly over AM and NM for improvement in plant height (Table 1; Fig. 2). No significant change was observed among Zn20 + NM and AM, but Zn20 + NM was significantly different over NM. However, Zn40 + NM remained significantly better over control (AM). Treatments Zn80 + AM, Zn100 + AM and Zn120 + AM significantly decreased plant height over AM. Same trend was also noted among Zn80 + NM, Zn100 + NM and Zn120 + NM over NM. The maximum increase of 15% and 25% in plants height was observed in Zn20 + AM as compared to AM and NM respectively.
### Table 1
Effect of different application rates of Zn with and without AMF on growth attributes of maize

| Treatment | Plant height (cm) | Stem girth (cm) | No. of leaves |
|-----------|------------------|-----------------|---------------|
|           | AM | NM  | AM | NM | AM | NM |
| Control   | 160c | 147d | 2.7ab | 2.6bc | 9.3c | 8.3de |
| Zn20      | 184a | 158c | 2.8a  | 2.7 cd | 12.6a | 9.3c |
| Zn40      | 182ab | 176b | 2.6b  | 2.5b  | 12.0a | 11.0b |
| Zn60      | 149d | 136e | 2.5d  | 2.4e  | 9.1 cd | 8.5cde |
| Zn80      | 138ef | 118gh | 2.4e  | 2.3f  | 9.0 cd | 8.0ef |
| Zn100     | 132ef | 113h | 2.3f  | 2.1 g  | 8.3de | 7.3 fg |
| Zn120     | 124 g  | 111fh | 2.2 g  | 1.9 h  | 7.3 fg | 7.0f |
| AM        | *** | *** | *** | *** |
| Zinc      | *** | *** | *** | *** |
| AM*Zn     | * | *** | ** |

* (p ≤ 0.005), ** (p ≤ 0.01), *** (p ≤ 0.001)

### 3.3. Stem girth

Different application rates of Zn with (AM) and without AMF (NM) significantly affected the stem girth of maize. Treatment Zn20 + AM was significantly different over NM for increase in stem girth (Table 1; Fig. 3). No significant change was observed among Zn20 + AM, Zn40 + AM and AM for stem girth. Treatments Zn80 + AM, Zn100 + AM and Zn120 + AM significantly decreased stem girth over AM. Same trend was also noted among Zn80 + NM, Zn100 + NM and Zn120 + NM over NM. Maximum increase of 7.69% in stem girth was observed in Zn20 + AM over NM.

### 3.4. Number of leaves

With (AM) and without AMF (NM), various application rates of Zn significantly affected the number of leaves of maize. Application of Zn20 + AM and Zn40 + AM remained significantly better over AM and NM for increase in number of leaves (Table 1; Fig. 4). A significant change was also observed among Zn20 + AM, Zn40 + AM over AM for number of leaves. Treatments Zn100 + AM and Zn120 + AM significantly decreased number of leaves over AM. Same trend was also observed among Zn100 + NM and Zn120 + NM over NM. Maximum increase of 35.4% and 51.8% in number of leaves was recorded in Zn20 + AM over AM and NM respectively.
3.5. Cobb weight

Effect of various application rates of Zn with (AM) and without AMF (NM), differed significantly for cob weight of maize. Application of Zn20 + AM was significantly different over AM and NM for increase in cob weight (Table 2; Fig. 5). Treatments Zn20 + NM and Zn40 + NM was significantly better for cob weight from NM. A significant change was also observed among Zn20 + AM, Zn40 + AM and Zn60 + AM over NM for cob weight. Treatments Zn80 + AM, Zn100 + AM and Zn120 + AM significantly decreased cob weight over AM. Same trend was also observed among Zn80 + NM, Zn100 + NM and Zn120 + NM over NM. Maximum increase of 4.39% and 12.8% in cob weight was recorded in Zn20 + AM over AM and NM respectively.

Table 2
Effect of different application rates of Zn with and without AMF on growth attributes of maize

| Treatment | Cob weight (g) | Cob length (cm) | 1000-grain (g) | Biological Yield (t/ha) |
|-----------|----------------|-----------------|----------------|-------------------------|
|           | AM             | NM              | AM             | NM                      |
| Control   | 227.6bc        | 210.6d          | 16.5a          | 15.2ef                  | 152d | 138 g | 4.17c | 3.79d |
| Zn20      | 232.6ab        | 222.6c          | 20.8a          | 16.4d                   | 168a | 143f | 5.93a | 4.30c |
| Zn40      | 237.6a         | 227.6bc         | 17.7c          | 19.5b                   | 163b | 158c | 5.21ab| 5.06b |
| Zn60      | 224.6c         | 214.6d          | 15.5e          | 14.5f                   | 158c | 158c | 5.00c | 4.88e |
| Zn80      | 211.6d         | 201.6e          | 15.5e          | 14.5f                   | 138 g | 146e | 4.76ef| 4.03gh|
| Zn100     | 203.3e         | 176 g           | 15.5e          | 14.5f                   | 132 h | 122i | 3.78ef| 16.13 h|
| Zn120     | 190f           | 139 h           | 14.5f          | 13.4 g                  | 128hi | 117j | 3.72 fg| 3.82 h |
| AM        | ***            | ***             | ***            | ***                     |
| Zinc      | ***            | ***             | ***            | ***                     |
| AM*Zn     | ***            | ***             | ***            | *                       |

* (p ≤ 0.005), ** (p ≤ 0.01), *** (p ≤ 0.001)

3.6. Cob length

Addition of various application rates of Zn with (AM) and without AMF (NM), differed significantly for cob length of maize. Treatments Zn20 + AM remained non-significant over AM but significant over NM for increase in cob length (Table 2; Fig. 6). Addition of Zn20 + NM and Zn40 + NM differed significantly for cob length from NM. A significant decrease was also noted among Zn40 + AM, Zn60 + AM, Zn80 + AM and Zn100 + AM over AM for cob length. Treatments Zn60 + NM, Zn80 + NM, Zn100 + NM and Zn120 +
NM also showed significant decline in cob length over NM. Maximum increase of 36.8% in cob length was observed in Zn20 + AM over NM.

### 3.7. 1000 grains weight

Different application rates of Zn with (AM) and without AMF (NM), remained significantly different for 1000 grains weight of maize. Treatments Zn20 + AM and Zn40 + AM remained significantly better over AM for increase in 1000 grains weight (Table 2; Fig. 7). Addition of Zn20 + NM, Zn40 + NM, Zn60 + NM and Zn80 + NM significantly enhanced 1000 grains weight of maize from NM. A significant decrease was observed among Zn80 + AM, Zn100 + AM and Zn120 + AM over AM for 1000 grains weight. Treatments Zn100 + NM and Zn120 + NM also showed significant decline in 1000 grains weight over NM. Maximum increase of 10.5% and 21.7% in 1000 grains weight was observed in Zn20 + AM over AM and NM respectively.

### 3.8. Biological yield

Effect of Zn variable application rates with (AM) and without AMF (NM), remained significantly different for biological yield of maize. Treatments Zn20 + AM and Zn40 + AM was significantly better over AM for increase in biological yield of maize (Table 2; Fig. 8). Treatments Zn20 + NM and Zn40 + NM also significantly enhanced biological yield of maize from NM. A significant decline in biological yield was observed where Zn80 + AM, Zn100 + AM and Zn120 + AM were applied over AM. Treatments Zn60 + NM, Zn80 + NM, Zn100 + NM and Zn120 + NM also gave significant decline in biological yield over NM. Maximum increase of 42.2% and 56.5% in maize biological yield was observed in Zn20 + AM over AM and NM respectively.

### 3.9. Photosynthetic rate

Effect of Zn variable application rates with (AM) and without AMF (NM), was significantly different for photosynthetic rate of maize. All the application rates of Zn significantly increased photosynthetic rates in maize over AM (Fig. 9). Treatments Zn20 + NM, Zn40 + NM, Zn60 + NM and Zn80 + NM also significantly enhanced photosynthetic rate of maize from NM. Treatments Zn120 + NM gave significant decline in photosynthetic rate over NM.

### 3.10. Transpiration rate

Application of Zn variable rates with (AM) and without AMF (NM), was significantly different for transpiration rate of maize. All the application rates of Zn significantly increased transpiration rate rates in maize over AM (Fig. 10). Treatments Zn20 + NM, Zn40 + NM, Zn60 + NM and Zn80 + NM also enhanced transpiration rate significantly in maize over NM. Treatments Zn120 + NM caused a significant decrease in transpiration rate over NM.
3.11. Stomatal conductance

Application of Zn variable rates with (AM) and without AMF (NM), was significantly different for stomatal conductance of maize. Addition of Zn20 + AM significantly enhanced stomatal conductance in maize over AM (Fig. 10). It was noted that stomatal conductance was decreased in Zn60 + AM, Zn80 + AM, Zn100 + AM and Zn120 + AM over AM. Treatments Zn20 + NM also enhanced stomatal conductance significantly in maize over NM. Application of Zn80 + NM, Zn100 + NM and Zn120 + NM caused a significant decrease in stomatal conductance over NM.

3.12. Total soluble protein

With (AM) and without AMF (NM), addition of Zn variable rates differed significantly for total soluble proteins of maize. Addition of Zn20 + AM and Zn40 + AM significantly improved total soluble protein in maize over AM (Fig. 10). It was observed that total soluble protein was decreased in Zn100 + AM and Zn120 + AM over AM. Treatments Zn20 + NM and Zn40 + NM also enhanced total soluble protein significantly in maize over NM. Addition of Zn80 + NM, Zn100 + NM and Zn120 + NM caused a significant decline in maize total soluble protein over NM.

3.13. Pearson correlation and principal component analysis

Pearson correlation showed that maize growth attributes were positive and significant in correlation with gas exchange attributes and yield components affected by different application rates of Zn with and without AMF (Fig. 13). Principal component analyses showed that stomatal conductance, transpiration rate, total soluble protein and biological yield was more closely associated with Zn20 (Fig. 14B). Photosynthetic rate and AMF colonization was more closely associated with Zn60. All the growth, yield and gas exchange attributes were more closely associated with AMF (Fig. 14B). Chord diagram is clearly showing that on an average contribution, Zn20 + AM (9.79%) was best in the improvement of all the attributes of maize in the current study.

4. Discussion

Colonization of roots by AMF varies with external nutrient concentration. Application of Zn in Zn-deficient soil increase AMF root colonization percentage [35, 36]. Whereas, high Zn (80–120 mg kg\(^{-1}\)) supply reduced the AMF root colonization percentage of maize and results was agreed with Watts-William et al. [37]; Wang et al. [16]; Coccina et al. [15]. Lingua et al. [38] reported that at high concentration of soil Zn, AMF colonization was suppressed by reducing hyphal growth, preventing spore production and germination. High Zn concentration in roots, mediates two kinds of response, i.e. fungal and plant response. Alteration in fungal response includes reduced spore germination, spore life, density of spores, growth of hyphae and appressoria formation [39]. In plant response, high Zn upregulate plant defense system, alters hormonal balance and most important, it alters the root exudates composition [40, 41]. Little root colonization rate (20–40%) was also observed in non-inoculated plants because Thiophenate was not properly inhibit root colonization only reduced its growth [42]. Irrespective of AMF inoculation
high Zn supply negatively affect the plant growth parameters and yield as reported in previous studies [16, 37]. Inoculated plants showed improved maize growth. AMF have a nutrient threshold level below which it upregulates the nutrient transporter genes, and on above nutrient concentration, it imparts a protective effect on plants and increases plant growth [43]. A detailed study by Pellegrino et al. [44] reported that AMF inoculation increased 20% of plant yield than control. A similar result was also reported by Zhang et al. [45], that inoculation of *R. irregularis* increased 17% yield, and in another study of wheat, 24% increase in grain yield was observed at a high level of Zn [15]. Another study also reported high yield in *R. irregularis* inoculated plants even at 100 and 500 mg Zn/kg application [46]. AMF colonization rate was positively correlated with plant height, stem width, cob weight and length. Coccina et al. [15] described the reason behind this improved growth is increased P uptake by the plant as this was also clearly denoted in present study results. Application of Zn in deficient soil increased photosynthesis, transpiration and stomatal conductance rate [47]. It might be due to an increase in TSP contents which was also observed in the current study.

Our results were also supported by Liu et al. [48] he reported increased in photosynthetic efficiency by increasing chlorophyll a and b contents due to Zn application in Zn deficient soil [48]. Zinzala et al. [47] reported that above 80 mg Zn kg\(^{-1}\) soil application, all physiological parameters negatively affected due to the toxicity of Zn in plant tissues [47]. We observed a reduction in net photosynthetic rate (A) and transpiration rate (E), due to reduced stomatal conductance. However, no visual symptoms of Zn toxicity were observed in our study [49]. Sagardoy et al. [50] reported that Zn toxicity reduced the stomatal conductance (gs) severely (76%), which limits other related physiological and chemical process [50]. They also studied that excess Zn leads to alteration in the physical structure of mesophyll cells and stomata. Reduced photosynthesis was also due to the restricted activity of carbonic anhydrase enzyme [50]. Vassilev et al. (2011) studied that excess Zn in plant tissues, reduced Mg\(^{2+}\) uptake and replace Mg atom, which distorts chlorophyll structure and result in decreased photosynthetic rate [49]. Inoculation of AMF, improved total soluble protein contents in maize [51, 52]. Results of our study are in accordance with Nguyen et al. [52]. In soil with low Zn, AMF increases carbonic anhydrase enzyme activity and enhances photosynthesis. In contrast, soil with high Zn, AMF imparted protective effect on plants by mitigating its toxicity effect [52]. In this study, results showed that AMF inoculated plants showed a better physiological response at high soil Zn as compared to mock-inoculated and results are in line with Wang et al. [16].

5. Conclusions

Application of Zn20 + AM has more potential to improve the growth attributes of maize over AMF cultivated in Zn deficient conditions. A significant increase in yield attributes of maize through addition of Zn20 + AM validated the efficacious functioning of combined used of Zn and AM. Maximum improvement in photosynthetic rate, gas exchange attributes and stomatal conductance over AM signified the imperative role of Zn20 + AM. Higher application rates of Zn, i.e. 80, 100 and 120 induced adverse effects on maize growth, yield and gas exchange attributes with AM and NM. It is recommended to apply Zn20 + AM for the achievement of better growth, yield of maize in Zn deficient soils. However,
more investigations are suggested for optimization of Zn application rate from control (0 20 mg kg⁻¹) to 20 mg kg⁻¹.

Declarations

Author Contributions: “Conceptualization, A.S.; M.A.A; methodology, M.A.; software, S.D.; validation, S.D., M.A.A. and N.A.; formal analysis, A.S.; investigation, A.S.; resources, M.A.A.; data curation, A.S.; writing—original draft preparation, M.A.A.; S.D.; R.D.; S.F.; writing—review and editing, M.A.A.; S.D.; R.D.; S.F.; supervision, M.A.A.; funding acquisition, Y.Y.

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