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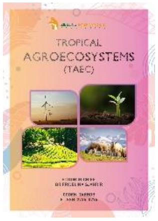

RESEARCH ARTICLE

EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND NITROGEN SOURCES ON GROWTH AND NUTRIENT UPTAKE OF MAIZE PLANT IN SOIL OF MINNA.

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ABSTRACT

A screen house experiment was carried out at Federal University of Technology, Minna, in the cropping season of 2021 to determine the response of maize to different sources of Nitrogen and AMF in soil of Minna. Soil samples were obtained from Teaching and Research Farm, Gidan Kwano Campus at a depth of 0 – 15cm with the aid of sterilized auger. Prior to treatment, seeds of AMANA-1 Maize variety were sown at the rate of 4 seeds per pot and later thinned to two seedlings after one week of planting. At day 1 of planting, pots were treated with basal application of 200 ml hydroponic nutrient solution per pot and seedlings were watered daily except when hydroponic nutrient solution was applied. Plants were then treated as follows: Soil N at the rate of 0 Kg N ha^{-1} (control), Poultry N at the rate of 60 Kg N ha^{-1} (0.22 g per 2 Kg soil) and Urea N at the rate of 60 Kg N ha^{-1} (0.1g per 2 Kg soil)] and 3 AMF sources [No AMF (control), Native AMF and Elite AMF species (*Glomus intaradices*) inoculated at the rate of 1 ml per plant]. Treatments were replicated 3x and fitted to a Completely Randomized Design (CRD). Plants were harvested at 6 weeks after planting. Data generated were subjected to Analysis of Variance (ANOVA) and the means were separated using Least Significant Difference (LSD) where significant difference were observed at 5% probability level. Result obtained showed that apart from shoot N and P, growth of maize was not significantly affected by the interaction between Nitrogen and AMF sources. Shoot P was highest when soil N (control) interacted with no AMF or when poultry N interacted with no AMF implying that AMANA-1 is P efficient. Regardless of AMF sources, combinations with poultry N produced the highest shoot N content signifying that Poultry Manure supplied more nitrogen to the maize plant especially in association with *Glomus Interadices*. Therefore, if the farmer's choice is AMANA-1, there will be no need for inoculating maize with AMF to achieve significant increase in shoot P content but there will be a need for application of poultry manure if significant shoot N content must be achieved.

KEYWORDS

Maize, Nitrogen, Arbuscular, mycorrhizal, Fungi.

1. INTRODUCTION

With the global food demand increasing rapidly and much more in developing nations where crop lands and resources hardly contribute to an efficient crop production needed to meet such an urgent demand for food, there is a need to intensify agricultural production in a sustainable manner through use of efficient agro-biosystems which consider the entire agro ecosystem, bio-chemical diversity and their potential to mitigate the adverse impacts of low soil fertility, abiotic stress, pathogens, and pests (Tilman et al., 2011). Maize accounts for almost half of the calories and protein consumed, and one fifth of the calories and protein consumed in west Africa (Partey et al., 2014). Maize contains about 72% starch, 10% protein, and 4% fat, supplying an energy density of 365Kcal/100g, as compared to rice and wheat, but has lower protein content. Maize (*Zea mays*) belonging to the family poaceae take up only small proportion of Nitrogen (N) applied to soil due to rapid conversion into insoluble complexes and leaching (Narsian, and Patel, 2000; Malhotra et al., 2018). Roots of Maize plants form symbiotic association with Arbuscular mycorrhizal fungi (AMF), which increases the access of roots to a large soil surface area, causing improvement in nutrient uptake especially p and eventually plant growth (Bowles et al., 2015). Maize provides many of the B vitamins and essential minerals along with fiber, but lacks some other nutrients, such as vitamin B12 and vitamin C, and is, in general, a poor source of calcium, folate, and iron. Iron absorption, particularly the hemeiron present in maize, can be inhibited by some components of foods in the diet, such as vegetables, tea (e.g., oxalates), coffee (e.g., polyphenols), eggs (e.g., phosvitin), and milk (e.g., calcium).

In countries where anemia and iron deficiency are considered moderate or severe on public health problems, the fortification of maize flour and cornmeal with iron and other vitamins and minerals has been used to improve micronutrient intake and prevent iron deficiency (Ranum et al., 2019). Arbuscular mycorrhizal fungi are soil fungi that develop symbiotic associations with most plant species (Rodriguez and Sanders, 2014). Arbuscular Mycorrhizal Fungi (AMF) are also key microbial component of agro-systems giving that they are the most widespread symbiosis on earth (concerns more than 80% of terrestrial vascular plants) which is defined by a symbiotic relationship involving a bi-directional exchange of nutrients between the two organisms (Wang et al., 2017).

Nitrogen is very important in protein synthesis and photosynthesis in plant however nitrogen cycling in agroecosystems is heavily dependent upon arbuscular mycorrhizal fungi (AMF) present in the soil microbiome (Verzeauxa et al., 2017). These fungi develop obligate symbioses with

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various host plant species, thus increasing their ability to acquire nutrients. However, AMF are particularly sensitive to physical, chemical and biological disturbances caused by human actions that limit their establishment. For a more sustainable agriculture, it will be necessary to further investigate which agricultural practices could be favorable to maximize the benefits of AMF to improve crop nitrogen use efficiency (NUE), thus reducing nitrogen (N) fertilizer usage (Verzeauxa et al., 2017)

1.2 Statement of the Research Problem

Arbuscular mycorrhizal fungi are increasingly used in organic cropping systems to increase yields. Although maize is largely used in organic farming, there is little knowledge on the impact of maize on native mycorrhizal fungi (Giovannetti et al., 2014). The addition of N can inhibit the colonization of AMF in some species of cultivated plants, its depressive effect being greater than that of P (Szanser et al., 2016). However, fertilization experiments have shown that N, P, and potassium (K) all may limit soil microbial processes to some extent (Liu et al., 2016). In many of tropical pasture sites, strong limitation of N and P together has been demonstrated.

1.3 Justification of the Research

Nitrogen (N) deposition is a key factor that affects terrestrial biogeochemical cycles with a growing trend, , where shortage of available phosphorus (P) is particularly acute and P has become a major factor limiting plant growth and productivity. Arbuscular mycorrhizal fungi (AMF) establish a mutualistic symbiosis with plants, and play an important role in enhancing plant stress resistance. However, the response of AMF to the combined effects of N deposition is poorly understood (Chuyu et al., 2020).

1.4 Aim and Objectives of the Study

The aim of this experiment is to evaluate the response of maize to different sources of nitrogen and Arbuscular Mycorrhizal fungi.

The objectives are to:

- Determine the effect of Arbuscular mycorrhizal fungi and nitrogen sources on shoot and root biomass of maize.
- Evaluate the effect of Arbuscular mycorrhizal fungi and Nitrogen sources on shoot P and N

2. METHODOLOGY

2.1 Study Area

The soil for the screen house pot experiment was collected from Teaching and Research farm Gidan kwano, Federal University of Technology Minna, Niger State. The global positioning system (GPS) location of the sampled points in Gidan Kwano coincides with latitude $9^{o}31'6''$N to $9^{o}31'50''$N and longitude $6^{o}26'26''$E to $6^{o}27'5''$ E. Minna has a mean annual rainfall of about 1200mm with about 90% of the rainfall between June and August and mean daily temperature that rarely falls below 22^{o}C reaching a peak of 36^{o}C to 40^{o}C between November to December and February to March respectively.

2.2 Vegetation and Soil Description

Within the site there are arable lands where maize, rice, yam, soy bean are usually cultivated and trees like mango, shear butter,teak tree, African baobab, neem tree. Shrubs like wild strawberry and wild sunflower. Grasses that are predominant include spear grass, stubborn grass and elephant grass. The soils of Minna are Alfisols developed from basement complex rocks ranging from shallow to very steep soils overlying deeply weathered gneisses and magnetite with some underlain by iron pan to varying depth.

2.3 Soil Sampling and Analysis

Soil samples were collected with the aid of an auger diagonally from 10 points at a depth of 0 – 15 cm within a plot. Before collecting the samples, soil auger was sterilized with methylated spirit to avoid contamination from previous soil sampling. The samples were collected in bags, bulked and mixed properly to obtain a composite soil sample. A sub-sample (10 grams) of the composite soil was kept in the refrigerator for AM fungi studies.

The other portion was air dried, carefully crushed and passed through 2mm sieve to remove gravels and roots for physical and chemical properties analysis according to the method of (ISRIC/FAO, 2002) as follows:

Soil particle size distribution was determined by Bouyoucos hydrometer method, Soil pH in 1:2.5 soil to water and $CaCl_2$ suspension with a glass electrode pH meter; soil organic matter using the Walkley and Black wet oxidation method, Total nitrogen by kjeldhal method, exchangeable bases was extracted with 1N NH_4OAC and phosphorus was extracted using Bray P-1 method and was determined using absorption spectrophotometer.

2.4 AMF Liquid Carrier Preparation

Glucose solution (5g: 250 ml distilled water) was autoclaved for 15mins in slant bottle. After cooling, content was inoculated with spores and incubated at 27 ºC for 3 days.

2.4.1 AMF Spore Count

AMF spore count was done using New Plate Method as follows: (Mosse and Bowen, 1968)

- One milliliter of AMF liquid was added to 9 mls of sterile distilled water and thoroughly mixed.
- Thereafter, 1 ml of carrier was placed on filter paper and was viewed under a dissecting microscope.

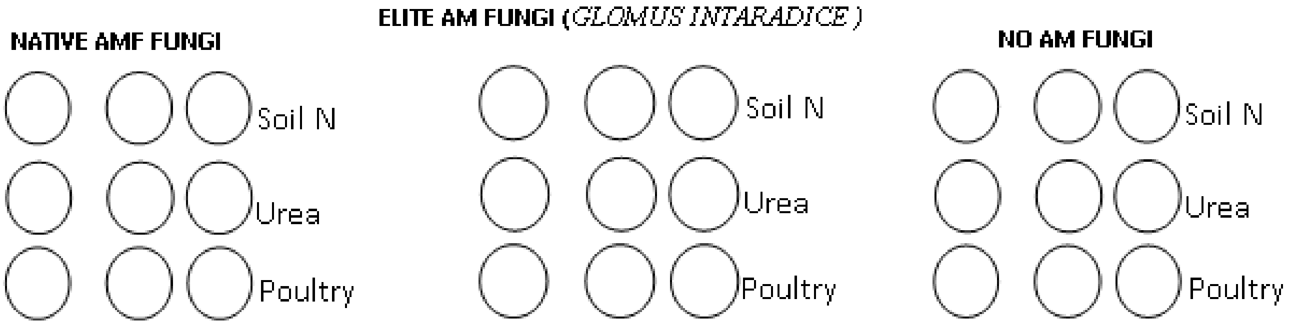
Coarse sand which was used for the pot experiment was washed several times using tap water to make sure that the sand was free from dissolved nutrients and sterilized using the autoclave at 121^{o}C for 20 minutes before transferring 2 kg per pot.

2.5 Seed Sowing and Crop Management

Seeds were sown at a depth of 2.5 cm, and at the rate of 4 seeds per pot after each pot had received a basal application of 200 ml hydroponic nutrient solution (a mixture of 10g NPK 20:10:10, 10g Ca (NO_3) and 5g Mg_2SO_4). Seedlings were later thinned to 2 per pot at a week after planting. Thereafter, pots were watered using 100 ml of distilled water daily unless when 200 ml hydroponic nutrient solution was applied at 3 days interval.

2.6 Treatments and Experimental Design

The experiment was a 2 factor experiment having AMF as a factor involving No AMF, Native AMF and Elite AMF species (*Glomus intaradices*) inoculated at the rate of 1 ml per pot and N source as the 2nd factor involving soil N at the rate of 0 kg N ha^{-1}, Urea N at the rate 60 kg N ha^{-1} (0.1g Urea) and Poultry N at the rate of 60 kg N ha^{-1} (0.22g of Poultry manure) per pot of soil. Treatments were replicated 3x and fitted to a Completely Randomized Design (CRD**).**



2.7 Harvesting and Data Collection

The harvesting was done at week 6 and the data was collected as follows:

2.7.1 Plant height (cm)

The height of the plant was measured from the base of the plant to the tip

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of the longest plant leaves using meter rule.

2.7.2 Shoot Biomass (g plant^{-1})

The shoot dry weight was obtained by weighing the shoots of 2 plants after oven drying for 3 days at 65 ^{o}C using top loading electronic balance and then dividing by 2.

2.7.3 Root Length (cm)

The root length was obtained by measuring the roots from the base of the plants to the tip of the longest root hair using meter rule.

2.7.4 Root Biomass (G Plant^{-1})

The root dry weight was obtained by weighing the roots of 2 plants after oven drying for 3 days at 65 ^{o}C using top loading electronic balance and then dividing values by 2.

2.7.5 Shoot P and Shoot N Content %

The shoot P was determined using Ascorbic Acid Method (Olsen and Sommers) 1982 and shoot N using Kjeldahl Method.

2.8 Data Analysis

All Data obtained was subjected to Analysis of variance (ANOVA) using Statistical Analysis System SAS, version 9.2 (2008). Least significant difference (LSD) was used to separate means where significant difference was observed at 5% probability level.

3. RESULTS

3.1 Selected Physical and Chemical Properties of The Soil

Table 1 shows the results of the physical and chemical properties of the soil used in carrying out the experiment. The textural class of the soil was sandy loam with the particle size distribution of clay, silt and sand as 170g/kg, 90g/kg and 740g/kg respectively. The pH of the soil was 6.36 which is moderately acidic soil reaction. The Available phosphorus of the soil was 4.00mg/kg which is low according to the rating of Esu (1991). Organic carbon (OC)of the soil was 6.75g/kg and Total nitrogen (TN) of the soil was 2.30g/kg showing that the soil was low in OC and moderately high in TN.

3.2 Main Effect of Nitrogen and AMF on Growth of Maize

3.2.1 Plant Height (cm)

The effect of N source and AMF on the plant height of maize is shown on Table 2. Plant height differed significantly among the N sources. The soil N (control) produced the tallest plants than the application of urea N and poultry N. Plant height was not significantly different among the AMF treatments.

3.2.2 Shoot Biomass (g plant^{-1})

The effects of N source and AMF on the shoot dry biomass of maize is shown on Table 2 N sources affected the shoot dry biomass significantly. The soil N (control) produced heavier shoot than the application of urea N and poultry N. Shoot biomass was not significantly different across all the AMF treatments.

3.2.3 Root Biomass (g plant^{-1})

The effect of N sources and AMF on the root dry biomass of maize is shown on Table .2. Root biomass was not significantly different among the N sources. The application of native AMF had a significantly different root biomass compared with No AMF and elite AMF treatments respectively. Consequently, the application of native AMF produced the heaviest dry roots, followed by No AMF treatment or treatment with elite AMF.

3.2.4 Shoot Nitrogen Content

The effect of N source and AMF on shoot N content of maize is shown on Table 2. N source affected shoot N content of maize significantly, such that the application of poultry N produced significantly the highest shoot N content than soil N (control) and urea N in that order. The application of AMF had a significant effect on the shoot N content of maize. The application of elite AMF recorded significantly highest shoot N content statically similar with maize receiving no AMF while the application of native AMF recorded the lowest shoot N content in this study.

3.2.5 Shoot Phosphorus Content

The effects of N source and AMF on shoot phosphorus content of maize is shown on Table 2. Shoot phosphorus content was not significantly different among the nutrient source in this study. Soil N (control) and the application of poultry N recorded significantly similar highest shoot phosphorus content than the application of urea N which recorded the lowest shoot P content. The pot with No-AMF recorded the highest shoot P content than native and elite-AMF (*Glomus intaradices*) which had similar lowest shoot P content in this study.

3.3 Effect of Nitrogen and AMF Interaction on Shoot Phosphorus and Nitrogen Content of Maize

3.3.1 Shoot N Content as Affected by N And AMF Interaction

The interaction effect between N sources and AMF on shoot N content is shown on Table 2.1. The combination of poultry N and elite AMF produced the highest shoot N content similar to the combination of urea N and No AMF compared with the combination of soil N and No AMF.

3.3.2 Shoot P Content as Affected by N And AMF Interaction

The interaction effect between N sources and AMF on shoot phosphorus content is shown on Table 2.2. Combination of soil N and No AMF, poultry N and No AMF produced significantly similar highest shoot P content. These shoot P contents were not significantly higher than the shoot contents of maize plants treated with a combination of soil N and native AMF.

4. DISCUSSION

The sandy loam textural class of the soil was because the particle size distribution of sand, silt and clay was 740g/kg, 90g/kg and 170g/kg respectively. The pH of the soil was 6.36 which is moderately acidic and suitable for the availability of soil nutrients. The Available phosphorus of the soil was 4.00 mg/kg which is low (Esu, 1991). And justifies the need to improve phosphorus uptake through AMF associations. Organic carbon (OC) of the soil was 6.75g/kg which is rated low and typifies marginal soils of the Nigerian savanna. Total nitrogen (TN) of the soil was 2.30g/kg which is high implying that the soil had previously received high doses of inorganic nitrogen fertilizers.

Table 1: Selected Physical and Chemical Properties of the Soil

Soil properties	Value	Rate
Particle size Distribution (g/kg)		
Sand	740	
Silt	90	
Clay	170	
Textural class	Sandy loam	
pH in 1:2.5 soil to water	6.36	Moderately Acidic
Available Phosphorus (mg/kg)	4.00	Low
Organic Carbon (g/kg)	6.75	Low
Total Nitrogen (g/kg)	2.30	Moderately High

The highest shoot phosphorus content, highest shoot nitrogen content and heaviest roots produced with the application of poultry N could be attributed to the availability and supply of phosphorus and nitrogen by the poultry N source throughout the growing period of the maize plant. Increased nutrient uptake efficiency in plants was due to the availability and release of nutrients by poultry manure throughout the growing periods of crops (Nweke and Nsanya, 2015). The heaviest shoots and tallest plants produced by the soil N source may be due to the availability of inherent nutrients in the soil used in this study.

The highest shoot phosphorus content recorded in the No-AMF treatment showed that the maize variety under-studied was P efficient and should be considered useful in P deficient soils. P use efficiency of Maize variety was evident in soils low in P (Bouzeriba et al., 2021a). This Maize variety (AMANA-1) was probably able to solubilize P by releasing phosphate enzymes from the root exudates which catalyze complex P forms in the soil, making them available for root uptake (Nadeem et al., 2022a)

The highest shoot nitrogen content produced with the application of *Glomus Interadices* could be attributed to the potential of the AMF to improve nitrogen supply to the infected roots of maize plant and hence the highest nitrogen uptake in this study.

The heaviest roots produced by the indigenous may be due to its ability to increase plant access to water and nutrients. AMF increased water uptake and improved water relations of maize plants during drought (Bouzeriba

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et al., 2021b). This implies that during water and nutrient shortages, AMF association elongates root length in search of nutrients and water, hence increasing their uptake. Therefore, root weight response of maize to AMF inoculation is an indication of their effectiveness. AMF is especially important for maize as it is the only grass known to produce less root biomass than shoot biomass (Hafiz et al., 2021a). In addition, heaviest root biomass produced by the native AMF could be attributed to the formation of arbuscules and hyphae in plant roots and rhizosphere, and hyphal network of plant roots which probably enhanced the access of roots to a large soil surface area, supporting increase in plant growth. The native AMF was effective in increasing carbohydrates storage at the roots and increasing the production of photosynthates at the leaves (Bowles et al., 2016). Hence resulting in increasing weight gains of the roots and shoots respectively (Hafiz et al., 2021b).

Table 2: Main Effect of P Sources and AMF Sources on Maize Growth and P Content

	Plant Height (Cm)	Shoot Fresh Biomass (g)		Root Dry Biomass (g)	Shoot N Content (%)		Shoot P (%)	
N Sources								
Soil N	99.39a	5.23a		3.07a	4.62b		0.31a	
Urea N	77.78b	3.67b		3.25a	5.08b		0.26a	
Poultry N	78.33b	3.64b		3.17a	5.99a		0.31a	
LSD(0.05)	10.04	0.91		0.99	0.6		0.03	
AMF (A)								
No AMF	85.39a	3.92a		2.74b	5.06ab		0.34a	
Elite AMF	84.78a	3.87a		2.74b	5.63a		0.28b	
Native AMF	81.33a	4.75a		4.01a	5.00b		0.27b	
LSD (0.05)	10.04	0.91		0.995	0.6		0.03	
Interaction								
N *A	NS	NS		NS	**		*	

Elite AMF = *Glomus intaradices*, N = Nitrogen. NS = not significantly different at $P > 0.05$. * = Significant at $P < 0.05$. ** = highly Significant at $P < 0.01$.

The highest shoot N content of 6.53 % obtained in the presence of elite AMF (*G Interadices*) and the 2nd highest shoot N content of 5.90 % recorded when poultry N was applied in the absence of AMF demonstrated that AMANA-1 responded highly to organic source of N than the inorganic source and that the moderately high soil N of 2.3 g kg^{-1} was probably insufficient for its N demand hence the need for additional external source, preferably the poultry source. Regardless of AMF source, inclusion of poultry N, improved N uptake significantly compared with the soil N (moderately high soil N) (Nweke and Nsanya, 2015).

The highest shoot P content of 0.35 mg recorded when soil N was applied in the absence of AMF and the 2nd highest shoot P content of 0.32 mg obtained in the presence of native AMF demonstrated that AMANA-1 was indeed P efficient and may not always need association with AMF to improve or increase P uptake (Nadeem et al., 2022b). The inherent soil P content of 4 mg Kg^{-1} was readily available for uptake by AMANA-1 through secretion of phosphatase enzymes contained in root exudates.

TABLE 3: Interaction of Nitrogen Source and AMF Source on Shoot N Content

		AMF	
	No AMF	**Elite AMF**	**Native AMF**
P Source			
Soil N	0.35e	5.67b	4.70d
Urea N	5.77ab	4.70d	4.77cd
Poultry N	5.90ab	6.53a	5.53bc
LSD (0.05)		0.7945	

Elite AMF = *Glomus intaradices*, N = Nitrogen

TABLE 4: Interaction of Nitrogen Source and AMF Source on Shoot P Content

		AMF	
	No AMF	**Elite AMF**	**Native AMF**
P Source			
Soil N	0.35a	0.26c	0.32ab
Urea N	0.31b	0.25c	0.23c
Poultry N	0.35a	0.32ab	0.24c
LSD (0.05)		0.0392	

Elite AMF = *Glomus intaradices*, N = Nitrogen

5. CONCLUSION

From the results of this present study, it is concluded that the application of poultry manure produced the highest shoot nitrogen content with *Glomus Interadices or without AMF inoculation.* The soil N recorded the heaviest shoot biomass and also the tallest maize plants. Soil N also produced the highest shoot P with or without AMF in native soil. The native AMF produced the heaviest root biomass of maize in this study.

RECOMMENDATION

The cultivation of AMANA-1 on Gidan Kwano soils, Minna will not need inoculation with AMF to achieve significant increase in shoot P content but there will be a need for application of poultry manure to achieve significant increase in shoot N content. Averagely, the inherent low P soil and moderately high N status of native soil was enough to significantly increase shoot P content of AMANA-1 above that of urea N and poultry N implying that AMANA-1 is P- efficient. Further studies should however be carried out to know how much of Nitrogen will be needed to sustain significant yield of AMANA-1 in the soil of Gidan Kwano, Minna.

DECLARATIONS

I, Bello, Lukeman Adeiza declare that this project work titled "Effect Of Arbuscular Mycorrhizal Fungi And Nitrogen Sources On Growth And Nutrient Uptake Of Maize Plant In Soil Of Minna" is a record of an original work undertaken and written by me under the guidance of Dr. A.O Uzoma and has not been presented previously for any other degree programme. Information derived from personal communication, published and unpublished works of others were duly acknowledged in the text.

STUDY LIMITATIONS

None.

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Competing Interests

None

Human and Animal Related Study

None

Ethical Approval

Not required

Informed Consent

I write on behalf of the authors that this research has been authorized for publication by my Supervisor, Dr Uzoma, A.O. Here is his contact email: ao.uzoma@futminna.edu.ng for verification.

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