

Response of Microbial Consortia Culture Inoculation to Soil Moisture Status, Proline and Yield of Sorghum (*Sorghum bicolor* L.)

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ABSTRACT

Article Info

Volume 8, Issue 4

Page Number : 05-13

Publication Issue

July-August-2021

Article History

Accepted : 25 June 2021

Published : 02 July 2021

Plant secondary metabolites play vital role in plant stress response. In this study we investigated root colonization of sorghum (*Sorghum bicolor*) infected by two different types of microbial consortia culture (MC1 & MC2) leads to alteration in the biosynthesis of secondary plant metabolites including phytohormones and osmolyte proline under drought stress. The promising microbial consortia procured from authentic sources (Department of Microbiology CRIDA, Hyderabad and that consortia culture contain mixture of effective beneficial microbes such as N-fixing bacteria, PGPR and mycorrhizal fungi). Application of microbial consortia culture through soil application and seed treatment to sorghum along with Recommended Dose Of Fertilizer (RDF) showed increased soil moisture content and chlorophyll pigments as compared to uninoculated controls as well as drought stressed plants. Proline content was increased in plants inoculated with microbial consortia under both normal as well as drought conditions. An obvious increase grain yield and dry matter yield through application of microbial consortia with soil application and seed treatments in normal and drought condition respectively.

Keywords : Microbial consortia, Drought, Soil moisture, Proline, Chlorophyll, Yield

I. INTRODUCTION

Drought is an abiotic stress resulting in devastation of agricultural crops and causing considerable losses in yield (de Vries et al., 2012). Drought stress is among the most destructive abiotic stresses that increased in intensity over the past decades affecting world's food security. Drought stress may range from moderate and short to extremely severe and

prolonged duration, restricting the crop yields (Austin, 1989; Pereira and Chavess, 1995; Bottner et al., 1995). Plant are constantly exposed to a wide range of environmental stresses which limit plant productivity. Over several centuries, breeding programmes have focused on generating crop species with enhanced productivity under suboptimal environmental conditions. Much work has been carried out in understanding the mechanisms behind plant

responses to various biotic and abiotic stresses (Staskawicz et al. 1995; Quartacci et al. 2000; Sgherri et al; 2000). Drought affect plant-water potential and turgor, enough to interfere with normal functions (Hsiao 2000) changing physiological and morphological traits in plants (Rahdari and Hoseini, 2012). Groth reduction under drought stress has been studied in several crops such as barley (Samarh, 2005), maize (Kamara et al., 2003), rice (Lafitte et al., 2007) and wheat (Rampino et al., 2006). Fresh weight and water content are common growth parameter that are affected by drought (Jaleel et al., 2009). Drought also induced free radical affecting antioxidant defences and Reactive Oxygen Species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radical resulting in oxidative stress. At high concentration ROS can cause damage to various levels of organization (Smirnoff, 1993), like initial lipid peroxidation, membrane deterioration and degrade proteins, lipids and nucleic acids in plants (Hendry, 2005; Sgherri et al., 2000; Nair et al., 2008). Nevertheless, under drought stress the decrease in chlorophyll content was symptom of photooxidation (Anjum et al., 2011; Rahdari et al., 2012). Millions of microbes inhabit plant root system forming a complex ecological community that influences plant growth and productivity through its metabolic activities and plant interactions (Berg 2009). The role of microorganisms in plant growth, nutrient management and biocontrol activity is very well established. These beneficial microorganisms colonize the rhizosphere/endo-rhizosphere of plants and promote growth of the plants through various direct and indirect mechanisms (Grover et al., 2011). Furthermore, the role of microorganisms in management of biotic and abiotic stresses is gaining importance.

In a country like India where large population depend on agriculture for their livelihoods, growth of agriculture assumes greater importance. The agriculture in India mainly relies on two monsoon

rains namely South West Monsoon and North East Monsoon. The recent failure in mansoon rain leaves 45% of the cropping area under drought (Indian Meteorological Department 2009). Thus, the identification of beneficial microorganisms which mediate drought resistance in crop plants assumes greater importance. Since three years there is continuous drought in Maharastra. Due to moisture stress there is significantly decreased in yield.

Microorganisms can help the crop to withstand in drought condition, by providing to the favorable environment to the crops. Thus keeping in view present study was conducted. There is necessity to use microbial inoculants formulations as an integral part of sustainable agricultural practices. These can be achieved by increasing and extending the role of microbial inoculation. Later, which help in minimizing the adverse environmental effect. (Vijaykumar Gagaraddi and GP Bramaprakash 2018). In the current study, the microbial consortia were tested to enhance drought resistance in sorghum (*Sorghum bicolor* L.) is an important cereal crop in India. Sorghum rank fifth among world cereals after wheat, rice, maize and barely (Sato et al., 2004 and Khalil, 2008). The crop is primarily grown in the warm dry climates of India, to used as food and fodder (Alagarswamy and Chandra, 1998). In India area under sorghum is 11.7 m ha⁻¹ with production 10.5 mt. In india, the share of sorghum out of total cropped area is about 7% and it contributes about 6.2% of total food grain production. Sorghum production in the country is largely concentrated in Maharashtra, Karnataka, Madhya Pradesh, Andrapradesh, Rajasthan, Tamil Nudu, Uttar Pradesh and Gujarat.

II. MATERIALS AND METHODS

The present experiment (2019) was laid out at VNMKV. Parbhani Center of All India Coordinated Research Project on Dry land Agriculture in a randomized block design, with seven treatments and

three replications under sorghum (*Sorghum bicolor* L.) with the objective to evaluate the performance of microbial consortia for drought tolerance and to assess their effect on soil moisture, proline, chlorophyll and yield under water stress condition. The application of microbial consortia were done at the time of sowing by seed treatment @ 100 g consortia powder per 10 kg of seed and by soil application @ 2.5 kg consortia culture powder ha⁻¹ through 50 kg FYM/ vermicompost. The treatments are T1: Seed treatment with consortia culture-1 (MC1), T2: Soil application of consortia culture-1 (MC1), T3: Seed treatment + soil application of consortia culture-1 (MC1), T4: Seed treatment with consortia culture-2 (MC2), T5: Soil application of consortia culture-2 (MC2), T6: Seed treatment + soil application of consortia culture-2 (MC2), T7: Absolute control.

Determination of chlorophyll from leaves of sorghum

Chlorophyll content was measured by SPAD meter (mg g⁻¹) at critical growth stages of sorghum.

Determination of proline

Proline content was determined as described by Bates et al. (1973). Briefly, Leaf tissue (0.5 g) for fresh plant leaf and (0.2 g) for dry plant leaf was homogenized in 5 ml of sulphosalicylic acid (3%) and centrifuged at 10,000g for 30 min. About 3 ml of the supernatant was taken in a test tube to which 2 ml ninhydrin reagent were added. The reaction mixture was boiled in a water bath at 100 °C for 30 min. After cooling the reaction mixture, four ml of toluene was added, and the sample was vortexed for 30 s; the upper phase containing proline was measured with a spectrophotometer at 520 nm using toluene as a blank. Proline content was expressed in µmol/g FW by using L-proline as a standard.

Determination of soil moisture

Soil moisture content (%) was estimated by gravimetric method by placing about 10g soil sample in oven at 105°C, and dry for 24 hours.

Determination of grain and dry matter yield of sorghum

Grain yield (Kg ha⁻¹) was recorded from each net plot and converted on hectare basis. For dry matter yield the plant were uprooted for dry matter study, excluding root system and were air dried under sun light for eight days and subsequently dried in the thermostatic oven at 64°C till they were completely dried. The final constant dry weight was recorded as total dry matter accumulation per plant and per hectare yield was calculated.

III. RESULT AND DISCUSSION

Effect of microbial consortia inoculation on chlorophyll content in sorghum Chlorophyll content in sorghum, indicated significant effect of microbial consortia in flag leaf and dough stage of sorghum crop. The chlorophyll content ranged from 4.90 – 7.06 mg g⁻¹ at flag leaf stage and 4.80 – 6.20 mg g⁻¹ at dough stage in sorghum crop. The significantly highest chlorophyll content 7.06 mg g⁻¹ was noticed at flag leaf stage and 6.20 mg g⁻¹ at dough stage of sorghum crop with application of microbial consortia culture-C2 through seed treatment + soil application (T6). The treatment T3 (seed treatment + soil application of consortia culture- C1) was found to be at par with T6 found 6.40 mg g⁻¹ and 6.20 mg g⁻¹ chlorophyll content at flag leaf and dough stage respectively. An extreme drought event with high temperature which lead to dramatic reduction in primary productivity (Ciais et al., 2005) which resulted in reduced plant development and leaf wilting. Consortia inoculation increased osmoregulant and showed the ability to grow under low moisture condition (Mapell et al., 2012). Hence the chlorophyll content with addition

of microbial consortia through seed and soil application recorded highest chlorophyll content in leaf. Similar finding also reported by Raja and Anandham, (2020) in the or pink pigmented facultative Methyloph (PPPFM) @ 1: 100 dilution for 18 h have recorded higher germination, seedling vigor and chlorophyll content.

Table 1. Effect of microbial consortia inoculation on chlorophyll content in sorghum

Treatments	Total Chlorophyll (mg g ⁻¹)	
	Flag leaf	Dough Stage
T ₁ : Seed treatment of consortia (C ₁)	5.16	5.16
T ₂ : Soil application consortia (C ₁)	5.46	5.36
T ₃ : Seed treatment + Soil application of consortia (C ₁)	6.40	6.20
T ₄ : Seed treatment of consortia (C ₂)	5.63	5.33
T ₅ : Soil application of consortia (C ₂)	5.60	5.26
T ₆ : Seed treatment + Soil application of consortia (C ₂)	7.06	6.20
T ₇ : Absolute control	4.90	4.80
S.Em.±	0.42	0.29
C.D.at 5 %	1.32	0.92
C. V. %	12.81	9.40

Effect of microbial consortia inoculation on proline content in leaves under drought condition

There was significant changes in proline content of plant leave due to use of microbial consortia. The proline content in leaves during dry spell ranged between 0.31-0.86 µg g⁻¹ and at harvesting stage is 0.74-1.29 µg g⁻¹. The significant and highest proline content 0.86 µg g⁻¹ was noticed with the application of microbial consortia culture-C2, through seed treatment + soil application (T₆) during dry spell. Similarly the proline content in leaves was maximum 1.29 µg g⁻¹ at harvesting stage with use of microbial consortia culture-C2 through, seed treatment + soil application in T₆ in sorghum. The treatment T₃ showed the at par result to the treatment T₆. Accumulation of some compatible solute in plant during drought or salinity could make the relative cellular osmotic potential to retain the water absorption ability.

Proline is one of the compatible solute like those. The increased in proline content could induced the production against the osmotic stress generated by drought or salinity (Liu and Zhu, 1997). Similar results were reported by Kalindee and Borkar (2013) in sorghum seed bacterization with four rhizobacterial isolate viz., *Serratia marcescens* L1SC8, *Pseudomonas putida* L3SC1, *Enterobacter cloacae* L1CcC1 and *Serratia marcescens*. L2FmA4 were found beneficial to mitigate drought stress effect in sorghum. This was attributed to increase in osmolytes i.e. proline accumulation.

Table 2. Effect of microbial consortia inoculation on proline content in leaves under drought condition

Treatment	Proline content (ug g ⁻¹)	
	Dry spell	Harvest
T ₁ : Seed treatment of consortia (C ₁)	0.76	0.84
T ₂ : Soil application consortia (C ₁)	0.69	1.03
T ₃ : Seed treatment + Soil application of consortia (C ₁)	0.85	1.10
T ₄ : Seed treatment of consortia (C ₂)	0.79	0.96
T ₅ : Soil application of consortia (C ₂)	0.80	0.93
T ₆ : Seed treatment + Soil application of consortia (C ₂)	0.86	1.29
T ₇ : Absolute control	0.31	0.74
S.Em.±	0.05	0.06
C.D.at 5 %	0.17	0.18
C. V. %	12.79	10.56

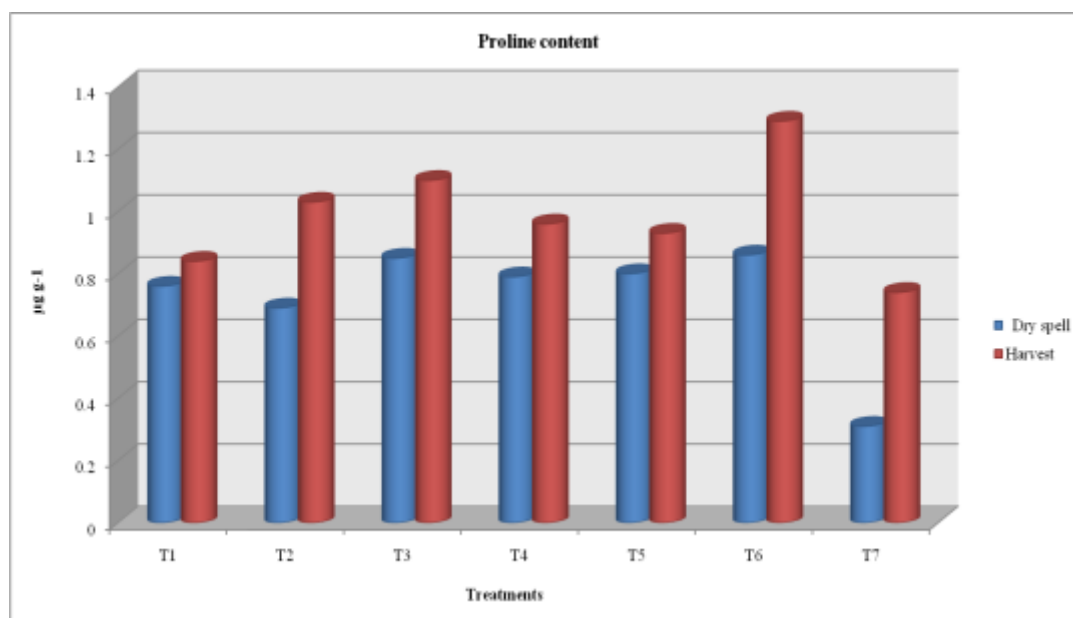


Fig 4.1 Effect of microbial consortia on proline content during dry spell and harvesting stage of sorghum.

Effect of microbial consortia inoculation on soil moisture content in sorghum

The data showed that the application of microbial consortia (C₁ and C₂) through seed treatment and soil application and both in conjunction has significant effect on moisture content of soil in various growth stages of sorghum. The moisture percentage during dry spell ranged from 21.19 to 24.45 percent. The highest percent moisture (24.45 %) was recorded in treatment T₆ during dry spell was noted in treatment T₇ of absolute control. The moisture content at flowering stage is in ranged from 25.43-29.98 %. The maximum moisture content (29.98%) was noticed in the treatment T₆ (seed treatment + soil application of microbial consortia culture-C₂). The treatment T₃ (seed treatment + soil application of consortia culture- C₁ along with RDF) which was at par to the treatment T₆. Moisture content at harvest stage of sorghum ranged from 15.43 to 19.61 percent. The highest moisture content at harvest stage was recorded with application of microbial

consortia through seed treatment + soil application of consortia culture- C₂. Followed by treatment T₂ (soil application of consortia culture- C₂) recorded the moisture content 18.57%. It was observed that Plant Growth Promoting Rhizobacteria (PGPR) could play a significant role in alleviation of drought stress in plants. These beneficial microorganisms colonize the rhizosphere/endo-rhizosphere of plants and impart drought tolerance by conserving soil moisture in soil and by producing exopolysaccharides (ESP), phytohormones, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, volatile compounds, inducing accumulation of osmolytes etc. in acquisition of drought tolerance (Sai Shiva Krishna et al., 2015).

Table 3. Effect of microbial consortia inoculation on soil moisture content in sorghum

Treatment	Moisture content (%)		
	Dry spell	Flowering	Harvesting
T ₁ : Seed treatment of consortia (C ₁)	23.72	26.99	17.09
T ₂ : Soil application consortia (C ₁)	23.14	25.77	18.57
T ₃ : Seed treatment + Soil application of consortia (C ₁)	24.32	27.81	18.28
T ₄ : Seed treatment of consortia (C ₂)	22.07	29.18	17.61
T ₅ : Soil application of consortia (C ₂)	25.00	26.32	15.41
T ₆ : Seed treatment + Soil application of consortia (C ₂)	24.45	29.98	19.61
T ₇ : Absolute control	21.19	25.43	15.43
S.Em.±	0.67	0.79	0.71
C.D.at 5 %	2.09	2.46	2.23
C. V. %	7.96	7.00	8.14

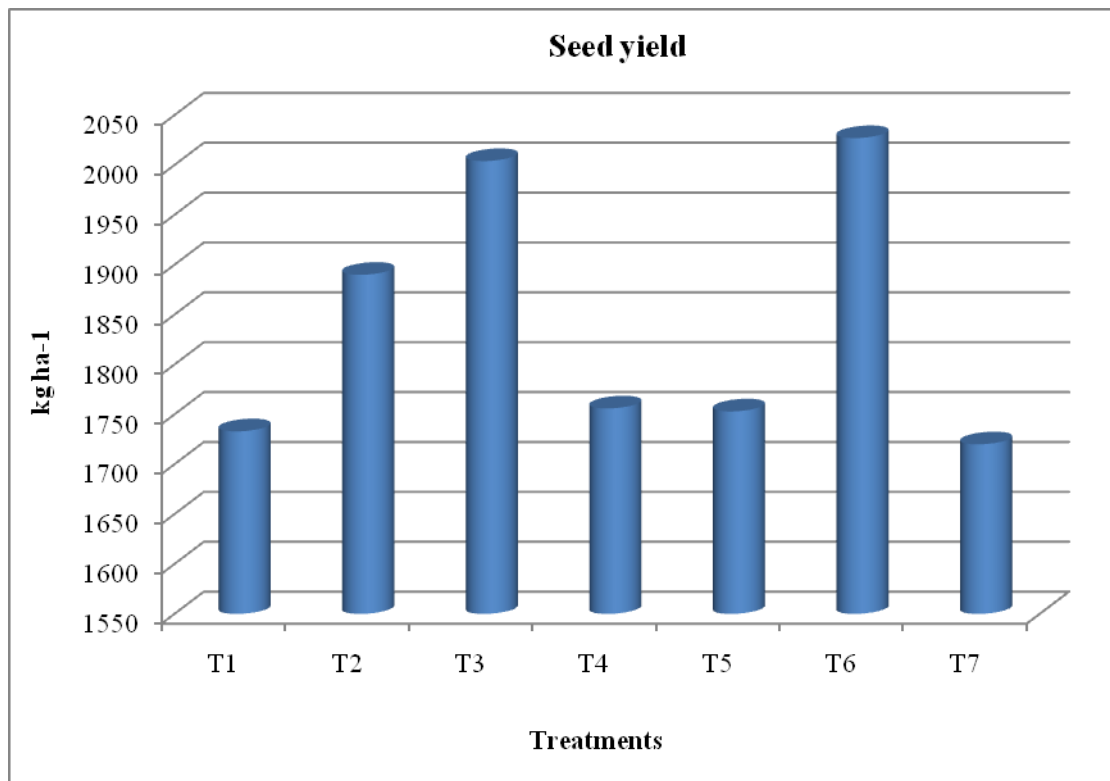
Effect of microbial consortia on yield and dry matter yield of sorghum under water stress condition

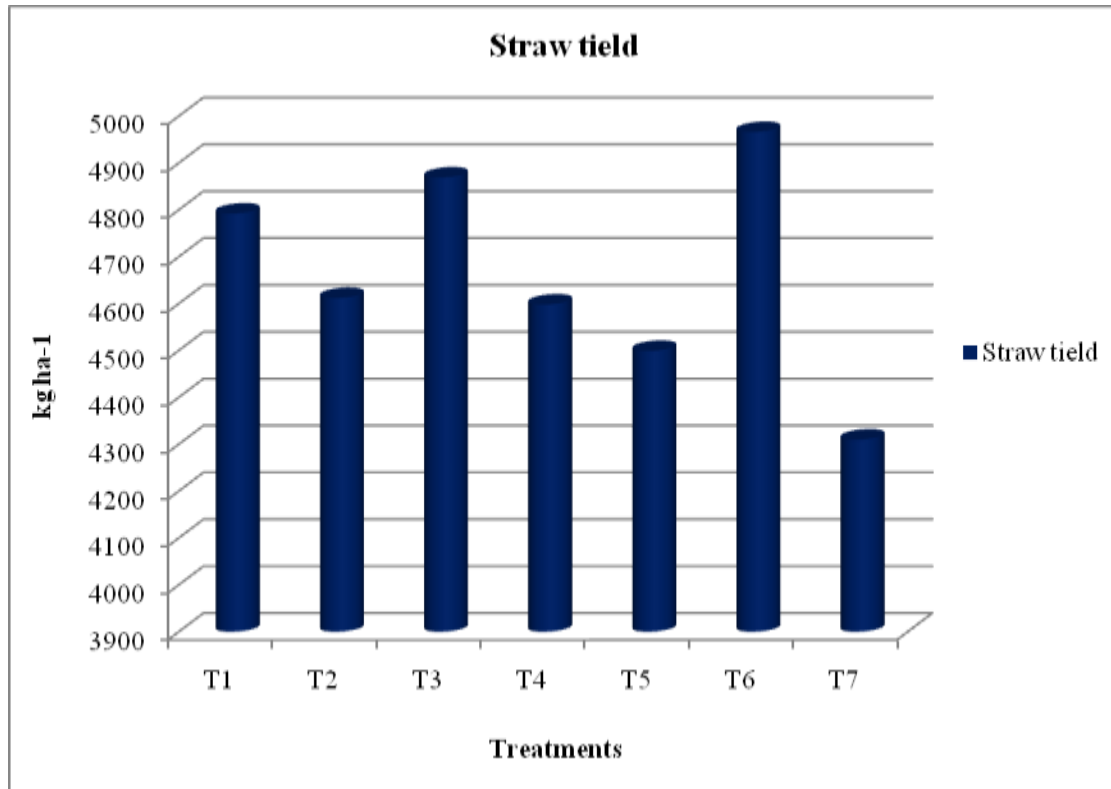
The significant and highest 2027 kg ha⁻¹ grain yield of sorghum recorded with application of treatment T₆ (seed treatment + soil application of consortia culture- C₂). Treatment T₃ and T₂ was found at par with T₆. Treatment T₃ (seed treatment + soil application of consortia culture- C₁) recorded 2004 kg ha⁻¹ grain yield. Similarly dry matter yield was significantly affected with the administration of different treatments of microbial consortia culture C₁ and C₂. The values of dry matter ranged between 4310 to 4966 kg ha⁻¹, sowing significant and maximum dry matter yield 4966 kg ha⁻¹ with administration of treatment T₆, which contain seed treatment in conjunction with soil application of consortia culture C₂. Treatment T₆ produced 15 percent more yield over control. Treatment T₃ was found at par with treatment T₆ and consortia culture- C₂ was found more effective to producing grain and straw yield of sorghum under dry spell of 16 days. Therefore production of proline, sugar and osmoregulant content in plant leaf and root (Shelvakumar et al. 2012) The production of proline and osmoregulants capable to withstand the water stress due to dry spell. These isolates were capable to increasing shoot and leaf biomass, shoot length and photosynthesis. (Kavya et al. 2015) Activities of drought challenged plant. Thus these bacteria contribute to improved plant adaption to drought through a water stress induced promotion ability and resulting in grain yield and straw yield of sorghum.

The higher concentration of consortia culture contributing to the formation of hydrophilic biofilm around the roots acting as an additional strength to protect the root system from soil hardness (Rossi et al. 2012; Xu et al. 2013).

Table 4. Effect of microbial consortia on yield and dry matter yield of sorghum under water stress condition

Treatment	Seed yield (kg ha ⁻¹)	Dry matter yield (kg ha ⁻¹)
T ₁ : Seed treatment of consortia (C ₁)	1733	4792
T ₂ : Soil application consortia (C ₁)	1890	4612
T ₃ : Seed treatment + Soil application of consortia (C ₁)	2004	4869
T ₄ : Seed treatment of consortia (C ₂)	1756	4598
T ₅ : Soil application of consortia (C ₂)	1753	4499
T ₆ : Seed treatment + Soil application of consortia (C ₂)	2027	4966
T ₇ : Absolute control	1720	4310
S.Em.±	66.26	100.59
C.D.at 5 %	206.43	313.39
C. V. %	9.23	8.73





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Cite this article as :

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