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Assessment Through Multilocation Trial for Unique Germplasm of *Hibiscus sabdariffa* L. with Special Reference to Flower and Fruit Colour

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ABSTRACT

Hibiscus sabdariffa L. commonly referred to as "Roselle" and "red sorrel" is a globally known annual herbaceous species of Malvaceae family holds utmost significance owing to its medicinal properties namely antioxidant, antimicrobial, and also for its proven utility as a natural colorant. The dark-red colored anthocyanin rich calyx has multi-purpose utilities including food, beverages, and medicines. The plant species shows its significant and promising potential in avenues of natural red colorants particularly water-soluble ones. Highly adaptive to varying soil conditions and humidity, the North Eastern states of India serve as a perfect cultivation house for this exquisite plant. In the present study 32 germplasm were planted for subsequent year *Kharif* 2021 and *Kharif* 2022 in randomized block design with three replicates. From the two years' evaluation 5 identified lines were subjected to multi-locational trials (MLT) along with one check variety named as local variety comprising of four different locations of NE India during *Kharif* 2023. Diverse climatic conditions of this region are responsible for the formation of new genotypes with varying chemical compositions. For this purpose, the MLT was performed at four different locations of North East India to confirm their stability performance. The main objective was to carry out a morphological and qualitative data analysis of *H. sabdariffa*. Plant height, colour of flower, colour of fruit, number of fruits per plant, average fruit weight and stem colour were evaluated. The qualitative and quantitative results demonstrate the identified lines were superior to that of the local (check) variety.

Keywords: Calyx, fruit size, flower colour, multilocation trial, roselle

Introduction

Hibiscus sabdariffa L. of the Malvaceae family is a herb commonly named as "red sorrel" or "roselle" in English (Betiku and Adepoju, 2013; Mahadevan and Kamboj, 2009). It is commonly known as Jamaica in Mexico and Spain, Bissap in Senegal, Karkade in Egypt, Wonjo in the Gambia, Congo in France, Zobo in Nigeria, Saudi Arabia and Sudan (Cisse et al., 2009; Mckay et al., 2009; Ramirez-Rodrigues et al., 2012). In India it is also known as Lal ambari, Gongura, Lal Mista, Patwa, Chukar in Bengali, Pulichchaikerai in Malayalam; Yerra Gogu in Telegu, Tengamora and Chutkiar in Assam (Gautam, 2004). *H. sabdariffa*, a perennial plant, is one of the most trafficked commercial and medicinal plants worldwide (Amlashi et al., 2020). *Hibiscus* genus includes more than 300 species of annual, perennial herbs, shrubs and trees (Wang et al., 2012). The *Hibiscus* species possesses various medicinal properties, among all the species one of them is *Hibiscus sabdariffa* (Orwa et al., 2014). It is distributed in subtropical and tropical regions of the world, popular for high fiber content and is used to make cloth and rope, cultivated in the late December to February (Ali et al., 2014).

In warmer countries like India, Malaysia, Indonesia, Sudan, Mexico, Saudi Arabia, Egypt, Philippines, Thailand and Vietnam *H. sabdariffa* is distributed (Wisetmuen et al., 2008). The main producing areas of Roselle are China, Mexico, Sudan, Thailand, Egypt (Carvajal-Zarrabal et al., 2012), native to tropical Africa and cultivated in Nigeria, tropical

America and West Indies (Inikpi et al., 2014). *H. sabdariffa* has red stems that can grow up to 3.5 meters tall, with deep-penetrating taproots. Its alternate leaves, which range from dark green to red, have long petioles measuring 3 to 5 inches (7.5-12.5 cm) in length. The flowers, known as Roselle, form in axillary or terminal racemes and feature white petals with a reddish center at the base of persistent columns, often used as food (Ali et al., 2014). The seeds are kidney-shaped, brown, and covered with minute hairs. The leaves grow alternately, have reddish veins, and are edible. At tender age, the fruits are found to be green and when it matures, it has five valves and each valve possess 3-4 seeds (Ali et al., 2014). Around the world, in different countries for many years this plant has been used as culinary and therapeutic resources (Ubani et al., 2010). Most commonly used in food industry as flavoring agents in puddings, cakes, as hot or cold beverages. In some parts of Africa, people ground the roselle seeds and add to meals (Duke and Du Cellier, 1993) whereas the calyx and leaves are served as vegetables (Mungole and Chaturvedi, 2011).

The red calyx is used to prepare ice creams, jams, jellies whereas, the leaves juice are used to improve the health and immune system (Idris et al., 2012). In traditional medicine, during summer the drink of *H. sabdariffa* provide relief by increasing blood flow to the skin surface (Mungole and Chaturvedi, 2011). The leaves in combination with ginger are used to suppressing high blood pressure (Ali et al., 2005). The ripe calyx is boiled in water can cure bilious attacks and ulcers, the flower is used as tonic, tea and proper kidney functioning. The leaves on heating can be applied in the feet for avoiding cracks and on boils (Ali et al., 2005). *H. sabdariffa* flower extract is caffeine free and helps in digestion and effective for inflammatory skin disorders (Wisetmuen et al., 2008).

Roselle is mostly known for the nutritional, medicinal purposes (Kolawole et al., 2014) and has various pharmacological properties like antioxidant (Mahadevan and Kamboj, 2009), antiviral (Idris et al., 2012), anticancer (Ubani et al., 2010), antimicrobial (Wisetmuen et al., 2008), hepatoprotective (Ologundudu et al., 2009), diuretic (Sandeep et al., 2010), immunomodulation, antiobesity (Carvajal-Zarrabal et al., 2012), hypocholesterolemic (Ali et al., 2005), antifungal (Ali et al., 2011), antipyretic (Bako et al., 2009), antianaemic (Khaghani et al., 2011), renoprotective (Kolawole et al., 2014), hypotensive, and antiurolithiatic (Kristen et al., 2014). Almost all parts of the plant contain bioactive compounds and inorganic minerals. The seeds are rich in carbohydrates, cellulose, fibers, and starch. Both the calyx and seeds are good sources of minerals such as potassium, magnesium, zinc, nickel, manganese, iron, phosphorus, calcium, sodium, aluminum, etc. (Rao, 1996).

Roselle seeds are a good source of edible oil (Ahmed and Hudson, 1979). The seeds on a dry weight basis are found to contain approximately 15% highly unsaturated triglycerides and small amounts of other lipid components (Nyam et al., 2009). In roselle seed oil, oleic and lenoleic acid are the major unsaturated fatty acids. High content of linoleic acid may provide a good source of essential fatty acids. Another major constituent found to be rich in the oil is alpha-tocopherol (Nyam et al., 2009). The seed also contain phytosterol compound such as desmethylsterol that has the ability to reduce dietary cholesterol absorption (Jones et al., 2000). The extract of *H. sabdariffa* possesses secondary metabolites such as tannins, saponnins, glycosides, phenols, and flavonoids. Due to the presence of flavonoid, anthocyanin, and polyphenol the flower acts as antioxidant (Camelo-Méndez, 2013). Two major anthocyanins are present in the plant namely cyanidin-3-sambubioside and delphinidin-3-sambubiside, which gives deep red pigment of the calyx and is the main contributors of antioxidant activity (Ali et al., 2005; Mungole and Chaturvedi, 2011; Camelo-Méndez, 2013). The compounds found in the calyx are galactose, galacturonic acid, gallic acid, gossypetin, hibiscetin, hibiscin, myricetin, protocatechuic acid, quercetin, rhamnose, and sabdaritrin (Zhang and Wang, 2007).

H. sabdariffa essential oil contains 17 compounds, constituting 99.8% of the total oil. In oil the compounds present are sesquiterpenes hydrocarbons, oxidized sesquiterpenes, diterpenes, aliphatic compounds, phenylpropanes and fatty acids. The main components of essential oils are the linoleic acid, hexadecanoic acid, and fatty acids. Minor components include tetradecanoic, methyl ester, heptadecanoic acid, methyl hexadecanoic acid, isophytol, and methyl linoleate (Inikpi et al., 2014). The calyx oil of *H. sabdariffa* is also rich in geraniol, menthol and undecalactone. Sterols include β-sitosterol, campesterol, avenasterol, cholesterol and clerosterol (Mungole and Chaturvedi, 2011). Roselle holds significant traditional and commercial value in both the food and household industries. Its diverse applications extend to the cosmetic, poultry, herbal, medical, and food industries, contributing to its constant demand. This suggests a promising future for *H. sabdariffa* in pharmaceutical and other sectors in the upcoming generations. Therefore, the present study emphasizes on the identification of elite germplasm of *H. sabdariffa* for the greater benefit of mankind.

Materials and methods

In the current study, a total of 32 germplasm were planted during *Kharif* 2021 in randomized blocks design (RBD) with three replications. The trial was replicated during the consecutive *Kharif* 2022. The plantation was done during May and harvested in the month of November for each of the year. The line to line spacing of 60 cm and plant to plant spacing of 45 cm was maintained. The germplasm was screened for the plant height (cm), colour of flower, fruit, number of fruits per plant, average fruits weight (g), and stem colour during *Kharif* 2021 and *Kharif* 2022. After two years evaluation, five unique lines were identified namely Jor Lab HS- 6, Jor Lab HS- 9, Jor Lab HS- 16, Jor Lab HS- 19, Jor Lab HS- 22. All identified lines along with one check variety named as local were planted in RBD with three replicated at four different locations of NE India (Jorhat, Bokakhat in Assam and Runne, Modai in Arunachal Pradesh) during *Kharif* 2023. All the morphological and quality data was reported as per standard protocol. For the colour data recording the Royal Horticultural Society (RHS) colour chart was taken as the standard reference.

Results and Discussion

Among the thirty-two germplasm, five elite lines of *H. sabdariffa* were identified namely, Jor Lab HS-6, Jor Lab HS- 9, Jor Lab HS- 16, Jor Lab HS- 19, Jor Lab HS- 22. The identified germplasm was subjected to morphological and qualitative data studies in MLT along with one local variety during *Kharif* 2023. During the study period the range of variation observed for plant height, flower colour, fruit colour, number of fruits per plant, fruit weight, and stem colour were recorded (Table 1). The colour of flower, fruit and stem were identified by the help of the Royal Horticultural Society (RHS) colour chart.

In Jor Lab HS-6, plant height ranged from 145-155 cm, flower colour was deep purplish pink, and fruit colour was vivid red, number of fruits per plant was found to be 55-59, fruits weight ranged from 4-6 g, stem colour was moderate purplish red. While, Jor Lab HS-9 exhibited 166-177 cm plant height, light purplish pink flower colour, vivid red fruit colour, 32-35 number of fruits per plant, 4-5 g was the range for fruit weight and stem colour was deep purplish red. Jor Lab HS-16 showed 183-190 cm range for plant height, deep purplish pink flower colour, deep purplish red fruit colour, range of 90-93 for number of fruits per plant, 6-7 g for fruit weight and deep purplish red stem colour. Meanwhile, Jor Lab HS-19 showed 187-193 cm range of plant height, pale yellowish pink flower colour, vivid red fruit colour, number of fruits per plant ranged from 74-77, 5-6 g for fruit weight and moderate purplish red stem colour. Jor Lab HS-42 exhibited plant height in the range of 210-220 cm, deep purplish pink flower colour, deep purplish red fruit colour, 103-108 number of fruits per plant, 7-8 g was the range for fruit weight and moderate purplish red stem colour respectively. Compared to the identified germplasm, the local variety showed 115-123 cm range for plant height, light purplish pink flower colour, moderate red fruit colour, number of fruits per plant ranged from 20-25, 3-4 g for fruit weight and grayish purple stem colour (Table 1).

Based on the MLT data for the average data for identified germplasm and local variety were tabulated (Table 2). From the data it was found that the germplasm Jor Lab HS-42 exhibited the highest average plant height of 218 cm, followed by Jor Lab HS-19 (192 cm), Jor Lab HS-16 (189 cm), Jor Lab HS-9 (175 cm) and Jor Lab HS-6 (153 cm). The local variety had a comparable average plant height (184 cm) similar to the identified germplasm. A similar trend was observed for the traits average fruit weight and average number of fruits per plant, where the average value of the local variety was below than that of the identified five germplasm. For the trait average fruit weight, the highest value was observed in Jor Lab HS-42 (7 g), followed by Jor Lab HS-16 (6.5 g), Jor Lab HS-19 (5.9 g), Jor Lab HS-6 (5.2 g) and Jor Lab HS-9 (4.1 g) which was higher than the local variety (3.6 g). Similarly, for the trait number of fruits per plant the highest value was observed in Jor Lab HS-42 (105) followed by Jor Lab HS-16 (91), Jor Lab HS-19 (75), Jor Lab HS-6 (58) and Jor Lab HS-9 (35) which was again higher than the local variety (30).

Considering the colour aspect, variation was observed for the traits flower colour, fruit colour and stem colour where RHS N66-D indicate deep purplish pink colour, RHS 65-B indicate light purplish pink colour, RHS-27-D indicate pale yellowish pink colour, RHS 62-C indicate light purplish pink colour, RHS 46-B indicate vivid red, RHS 59-B indicate deep purplish red, RHS N45-D indicate moderate red, RHS 184-C indicate moderate purplish red, RHS 71-A indicate deep purplish red and RHS N77-A indicate grayish purple. The colour of the flower for Jor Lab HS-6, Jor Lab HS-16, Jor Lab HS-42 was deep purplish pink (RHS N66-D), for Jor Lab HS-9 the colour was light purplish pink (RHS 65-B) and for Jor Lab HS-19 the colour was pale yellowish pink (RHS-27-D) compared to light purplish pink (RHS 62-C) for the local variety (Fig 1). Meanwhile the colour of the fruit for Jor Lab HS-6, Jor Lab HS-9 and Jor Lab HS-19 was vivid red (RHS 46-B), and for Jor Lab HS-16 and Jor Lab HS-42

the colour was deep purplish red (RHS 59-B) compared to moderate red (RHS N45-D) for the local variety (Fig 2). The stem colour for Jor Lab HS-6, Jor Lab HS-19, Jor Lab HS-42 was moderate purplish red (RHS 184-C), for Jor Lab HS-9 and Jor Lab HS-16 was deep purplish red (RHS 71-A) compared to grayish purple (RHS N77-A) for the local variety (Fig 3). Therefore, in this study, it revealed that all five germplasm (Jor Lab HS-6, Jor Lab HS-9, Jor Lab HS-16, Jor Lab HS-19 and Jor Lab HS-42) were found to be better than the check variety (local). Among the five selected elite germplasm Jor Lab HS-42 was the best performing.

A study conducted by Ilodibia et al. (2019) identified two varieties of *H. sabdariffa*: The red variety and the green variety. Morphological studies show no significant differences in their habits, and structure, but differ mainly in size and colour. Larger leaf area (94.25±0.310 cm^2) and petiole length (6.50±0.620 cm) was observed in the red variety as compared to the green variety. The leaf area and petiole length of the green variety are 53.95±0.400 cm^2 and 3.60±0.332 cm respectively. In a survey undertaken by Daudu et al. (2015), sixty Roselle accessions were collected showed that 41.7% of them were green in colour, and 31.7% had red calyxes. Among the accessions, 20% had deep red calyxes, while only 6.7% exhibited red and pink calyxes. Thus it can be said that aside from its medicinal properties, it is extensively utilized in food and beverage preparation. Another report by Sanders et al. (2020) revealed that based on a study on four genotypes of *H. sabdariffa* (African Green, Indian Red, Indian Variegated, and Thai Red) in New Jersey, the highest dry leaf weight was recorded in African Green Roselle (81.89 g), followed by Indian Variegated (79.94 g), Indian Red (74.23 g) and Thai Red (55.70 g) genotypes. Another report by Richardson and Arlotta, (2021) studied the yield of selected seven genotypes of *H. sabdariffa* near Washington, DC planted in three production systems (green roof, field row, high tunnel). From the study, it was found that in the field row the production of leaves was highest. According to Stevels (1990), Roselle plants containing anthocyanin pigments demonstrate greater resilience and tolerance to harsh environments compared to green varieties. From the present study, it can be clearly stated that the identified germplasm of *H. sabdariffa* holds promise for higher yield and further breeding program.

Conclusions

The present study revealed some important and unique morphological characteristics as well as excellent yield characteristics of some selected Roselle accessions. Considering the present result all the five germplasm (Jor Lab HS-6, Jor Lab HS-9, Jor Lab HS-16, Jor Lab HS-19 and Jor Lab HS-42) performed better than the local check variety. Moreover, considering the distinct morphological and colour variation of the selected germplasm all the germplasm are unique with the germplasm Jor Lab HS-42 performing with the highest yield across all the locations. Moreover, considering the different locations, Runne of Arunachal Pradesh was found to be best performing. Roselle has been reported to be used as a flavoring and coloring agent in food and beverage industry. It appears to be a good and promising source of water-soluble natural red dyes for use in the food and pharmaceutical industries. The overall data can be used as a useful tool in Roselle breeding to increase Roselle yield and improve accurate taxonomic characterization and plant species identification with great economic potential. This, in turn, can foster entrepreneurial opportunities and contribute to the upliftment of the socio-economic status of farming communities.

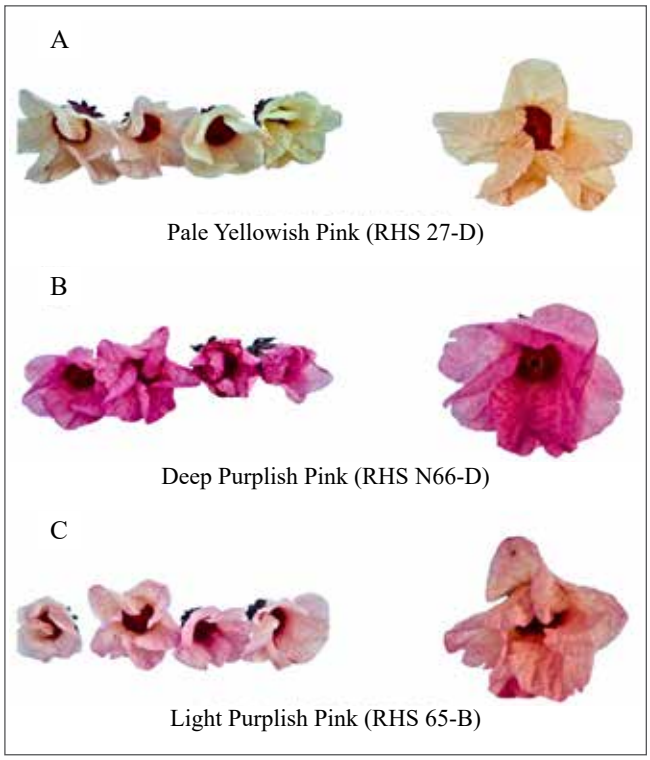

Figure 1. Different varieties of *Hibiscus sabdariffa* flower. (A) Jor Lab HS-19, (B) Jor Lab HS-6, Jor Lab HS-16, Jor Lab HS-42, (C) Jor Lab HS-9.

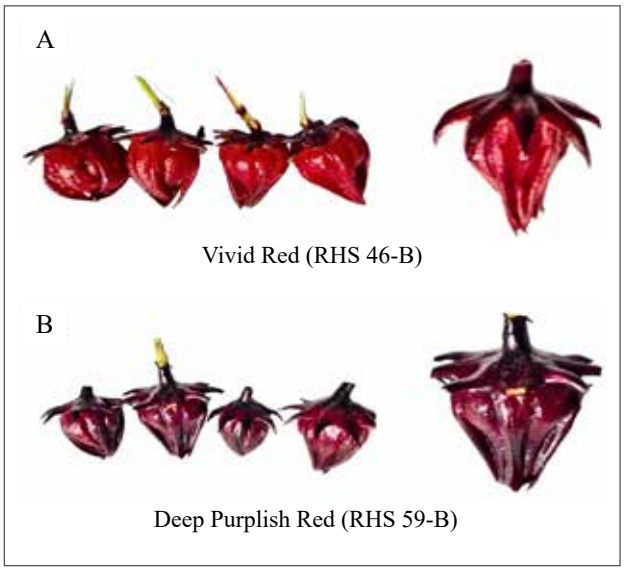

Figure 2. Different varieties of *Hibiscus sabdariffa* fruit (A) Jor Lab HS-6, Jor Lab HS-9, Jor Lab HS-19, (B) Jor Lab HS-16, Jor Lab HS-42.

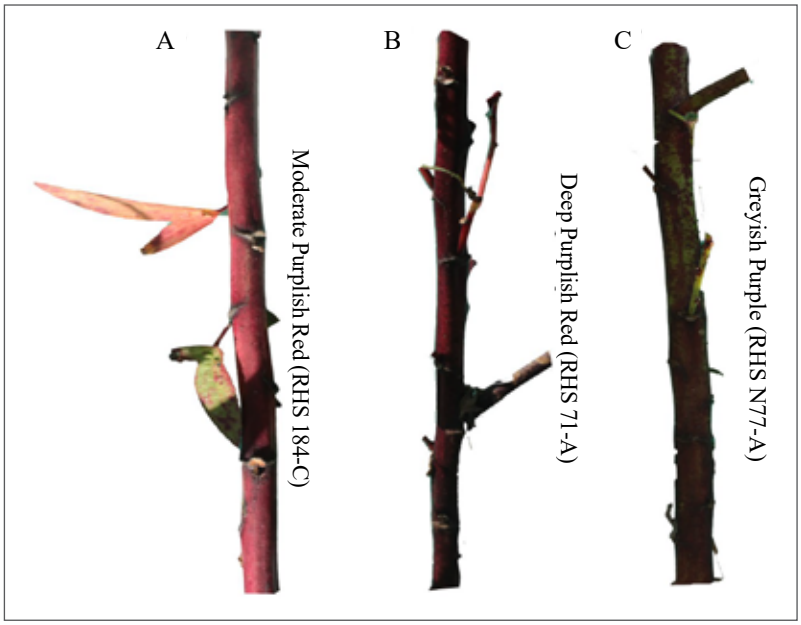

Figure 3. Different varieties of *Hibiscus sabdariffa* stem (A) Jor Lab HS-6, Jor Lab HS-19, Jor Lab HS-42, (B) Jor Lab HS-9, Jor Lab HS-16, (C) Check variety (Local)

Table 1. Range of traits taken for study among the germplasm of *Hibiscus sabdariffa* during *Kharif* 2021 and 2022.

Traits	Jor Lab HS-6	Jor Lab HS-9	Jor Lab HS-16	Jor Lab HS-19	Jor Lab HS-42	Check variety (Local)
Plant height (cm)	145-155	166-177	183-190	187-193	210-220	115-123
Colour of flower	Deep purplish pink	Light purplish pink	Deep purplish pink	Pale yellowish pink	Deep purplish pink	Light purplish pink
Colour of fruit	Vivid red	Vivid red	Deep purplish red	Vivid red	Deep purplish red	Moderate red
No. of fruits per plant	55-59	32-35	90-93	74-77	103-108	20-25
Fruits weight (g)	4-6	4-5	6-7	5-6	7-8	3-4
Stem colour	Moderate purplish red	Deep purplish red	Deep purplish red	Moderate purplish red	Moderate purplish red	Greyish purple

Table 2. Average morphological and qualitative data of identified germplasm along with the Check variety (Local) of *Hibiscus sabdariffa* during multilocational trial.

Pedigree	Plant height (cm)	No. of fruits per plant	Average fruits weight (g)	Colour of flower	Colour of fruit	Stem colour
Jor Lab HS-6	153	58	5.2	Deep purplish pink (RHS N66-D)	Vivid Red (RHS 46-B)	Moderate purplish red (RHS 184-C)
Jor Lab HS-9	175	35	4.1	Light purplish pink (RHS 65-B)	Vivid Red (RHS 46-B	Deep purplish red (RHS 71-A)
Jor Lab HS-16	189	91	6.5	Deep purplish pink (RHS N66-D)	Deep purplish red (RHS 59-B)	Deep purplish red (RHS 71-A)
Jor Lab HS-19	192	75	5.9	Pale yellowish pink (RHS-27-D)	Vivid Red (RHS 46-B)	Moderate purplish red (RHS 184-C)
Jor Lab HS-42	218	105	7	Deep purplish pink (RHS N66-D)	Deep purplish red (RHS 59-B)	Moderate purplish red (RHS 184-C)
Check variety (Local)	184	30	3.6	Light purplish pink (RHS 62-C)	Moderate red (RHS N45-D)	Grayish purple (RHS N77-A)
SD	19.49	27.53	1.25			
SE	4.59	6.49	0.29			
CV	10.52	21.93	13.25			

*SD= standard deviation, SE= standard error, CV= coefficient of variance

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