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EMERGING TRENDS IN LITTLE MILLET BREEDING: FROM TRADITIONAL METHODS TO GENOMIC APPROACHES

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ABSTRACT

Modern food and agricultural systems focus research and development on major crops while ignoring minor millets that are crucial for economic growth. Small-seeded cereal foods like little millet are an excellent way to supplement major staple foods. It is a short-duration crop that may be cultivated in drought-prone regions and has a higher nutritional content than some other major cereals. Its prevalence of pests and diseases is also lesser. Nowadays, farmers are more interested in cultivating millets because of their ability to adapt to a number of different agro-climatic zones and the need for greater research and development on these crops. This study provides detailed knowledge of the importance of, the global status of little millet germplasm, diversity, promising germplasm resources and as well as nutritional status. In addition, conventional breeding methods and genomic status expedite the development of climate-resilient and nutrient-dense varieties for sustainable agriculture, environment and healthy food systems. This review also examines breeding difficulties during research and the detailed molecular status of little millet varietal development.
Keywords : *Panicum sumatrense*, Traditional breeding, genomic approaches.

Introduction

Little millet (*Panicum sumatrense* L. Roth. Ex. Roemer and Schultes) is a staple small-seeded food crop and popularly known as kutki, sama, samo and vari belong to the family Poaceae and subfamily Pancoiedae. It is a multipurpose crop for food, fodder and bio-energy values. Moreover, it has better agronomic traits such as rapid growth with a short life cycle, well growth performance in both drought and waterlogged conditions (Doggett *et al.,* 1989). However, the production of little millet dropped from the 1950s to the early 21st century. But, in recent years the crop gaining much attention on production because of its high climate-resilient in adapting to the diverse agro-climatic zones and nutrient-rich components compared to other significant cereal food crops (Vetriventhan *et al.,* 2020). Globally, little millet is cultivated in an area of 0.26 m.ha with a production of 0.12 m.t (Bhat *et al*., 2018). In India, little millet was grown in an area of 2.34 lakh hectares with an annual production of 1.27 lakh tonnes and productivity of 544 kg ha^{-1}. In Andhra Pradesh, little millet is grown in an area of 7000 ha with a production of 3000 t and productivity of 354 kg ha^{-1} (Venkata Ratnam *et al*., 2019).

Although minor millets were superior to other cereals with many nutritional benefits, their utilization was limited because of low palatability, the coarseness of grain, and lack of diversified food preparations (Srilekha *et al*., 2019). Hence evaluation of the nutritional quality of millets would help to understand, diversifying the usage of millets in ensuring food, nutritional security in the ever-changing modern world.

Origin and Taxonomy

Little millet was derived from the Indian Peninsula (Weber and Fuller, 2007) so-called Indian millet, and specimens were collected from Sumatra (Indonesia) based on the name of the species (de Wet *et al*., 1983). Central India is the largest cultivation of little millet and is considered to be indigenous to the Indian subcontinent due to the presence of its wild ancestor *Panicum psilopodium* (de Wet *et al.,* 1983). The chromosomes of hybrids of *Panicum sumatrense* and *Panicum psilopodium* pair with only a single quadrivalent, representing that divergence between the two species may have developed initially through a single reciprocal translocation (Hiremath ***et al***., 1990). It can be cultivated in the temperate zones of Asia and also in the tropics of India, Srilanka, Pakistan, Indo-China, Nepal, and Myanmar (Hiremath ***et al***., 1990) more than 500,000 ha. In India, little millet is cultivated in Karnataka, Odisha, Andhra Pradesh, Maharashtra, Tamil Nadu, Madhya Pradesh, Uttar Pradesh, Jharkhand, Chhattisgarh, and Gujarat states (Kumar *et al*., 2017). It is an important catch crop in some tribal farms in India. Little millet is presently grown throughout India on about one million hectares. Currently, the crop is almost restricted to some hilly regions of India, while it provides good yields on marginal lands and contributes significantly to food security.

Little millet is highly self-pollinated C_4 plant with a basic chromosome number 9 (Tetraploid; 2n = 4x =36 AABB). It is a short-duration crop and less affected by environmental fluctuations while it is tolerant to poor agro-climatic conditions compared to other grain crops. Little millet is an annual herbaceous plant, which grows straight or with folded blades to a height of 30 cm to 1 m. The leaves are linear, sometimes with hairy lamina and membranous hairy ligules. As a food grain, it is suitable for people of all age groups so it is called wonderful millet (Prabhu *et al*., 2017).

Germplasm resources and utilization

Germplasm resources supply a pool of genes for the breeding of high-yielding, biotic and abiotic stress-resistant cultivars. Genetic diversity in crop cultivars is essential for sustainable agriculture, while germplasm provides the required variability for crop improvement. However, the narrow genetic base of cultivars leads to enlarging risk of vulnerability resulting in crop failure because of insect pests and disease epidemics or erratic climate effects (Vetriventhan *et al*., 2020). Globally, more than 3000 little millet germplasm accessions have been conserved in ex situ genebanks (Upadhyaya *et al*., 2015). Accessions of little millet majorly collected from Asia (2830), United States of America (226), Africa (7), and Oceania (1). However, India is well known for rich agro-biodiversity and has been a major contributor with 473 accessions of little millet (Upadhyaya *et al*., 2016). The largest germplasm collection (1253) of little millet was conserved at the National Bureau of Plant Genetic Resources (NBPGR)in India and Inter National Crops Research Institute for Semi-Arid Tropics (ICRISAT) gene bank conserved the 473 little millet germplasm accessions from various countries (Vetriventhan *et al*., 2020). Moreover, core collections representing a diversity of total collections of crops that have been developed for the finding of new sources of variation for biotic and abiotic stresses and for quality as well as important agronomic characteristics. In little millet, identified the trait-specific promising germplasm accessions *i.e*., 10 accessions for greater seed weight, 15 accessions for high grain yield, 15 for high biomass yield, and 30 for nutrient-rich accessions have been identified as well as 3 accessions for two or more nutrients.

Little millet accessions have been classified into two races based on panicle morphology, namely nana with 60-170 cm height and robusta with 120-190 cm (House *et al.,* 2000) and two sub-races per race; laxa and erecta for nana and laxa and compacta for robusta (de wett *et al*., 1983). The race of nana plants matured earlier than robusta but robusta plants give higher yields than nana. The two races of *Panicum miliare* or commonly known as little millet, is grown all over India and Sri Lanka and also cultivated in contiguous countries of India. In India, it is important to tribes of the Eastern Ghat Mountains and grown in combination with other millets (Hiremath *et al*., 1990). Till date, the antiquity of *Panicum sumatrense* cultivation is unidentified.

Nutritional status of little millet

The grain of little millet contains a good source of macro and micronutrients with high nutraceuticals and antioxidant properties. Little millet contains a higher amount of crude fiber, protein, carbohydrates, fat, iron, calcium, calories, minerals and vitamins (Table 2) are considered as to be essential for nutritional security compared to other cereals (Saleh *et al*., 2013). Moreover, the proteins contain a moderately balanced amino acid profile and are a good source of even the limiting amino acids, namely, methionine, cysteine, and lysine. It has lesser amount of carbohydrates than wheat and rice and has abundant quantities of Thiamin (Vitamin B1) which is not present in rice and wheat. Phytochemicals are rich in sama it contains excellent antioxidant properties, and have a low glycemic index to help prevent metabolic disorders like diabetes, cancer, obesity, etc (Fig. 1) (Srilekha *et al*., 2019). Little millet is high in fat, comprising majorly of healthy polyunsaturated fatty acids (PUFA). The flavonoids present in the little millet act as antioxidants and play many roles in the body's immune defense system. Little millets provides significant amount of bioactive nutraceutical components such as phenols, tannins, phytates, γ-aminobutyric acid (GABA), carotenoids and tocopherols, which are play an important role in health, aging and metabolic disease (Neeharika *et al.*, 2020). Henceforth they are considered as nutricereals. The grains can be preserved for more years without much loss and are least affectedly storage pests therefore the crop is recognized as an important reserve food crop during famines (Himanshu *et al.*, 2018).

Table 1 : Nutritional composition of little millet and other crops (per 100g)

Crops	Carbohydrates (g)	Protein (g)	Iron (mg)	Calcium (mg)	Crude Fiber (g)
Little millet	75.7	9.7	9.3	17	7.6
Foxtail millet	70.0	12.3	2.8	31	8.0
Rice	78.2	6.8	1.8	10	0.2
Wheat	71.2	11.8	3.5	41	1.2
Sorghum	72.6	11.0	3.4	13	6.7

Source: Habiyaremye *et al.* (2017).

Fig. 1 : Health benefits of little millet

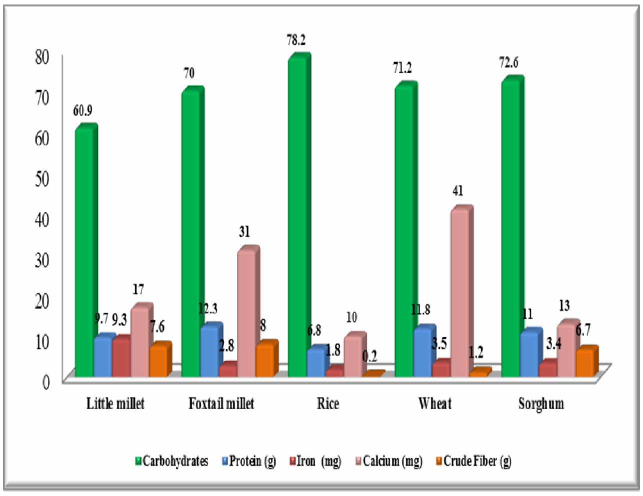

Fig. 2 : Nutritional composition of little millet and other crops (per 100g)

Breeding Status of Little Millet

At present, cultivating little millet varieties contains a significant amount of variability, but major bottleneck is lack of high-yielding ones. However, the development high yielding varieties are possible only through hybridization and selection in the segregating population. Hybridization is a process of a cross between two individuals from different populations that vary in one or more genetic traits (Harrison, 1990), and it can have immediate phenotypic consequences through the expression of hybrid vigor (Benjamin *et al.*, 2017). Hybridization is required for efficient exploitation of existing germplasm, production of breeding material to introgression the novel genes and to increase the genetic base. In general, yield and yield contributing characters are the most targeted traits in little millet improvement. Therefore, selection for yield *per se* has been the major source for improving productivity, but genotype ×environment interactions extremely influence these traits (Vetriventhan *et al.*, 2020). Therefore, assessing yield stability across multiple environments and investigating physiological traits associated with yield and adaptation are important for increasing the targeted yield levels. Most of the targeted traits in little millet are shoot fly resistance, non-lodging, and bold grain size of seed.

Moreover, it is well adapted to diverse climatic conditions and is less affected by major biotic and abiotic stresses. However, a few diseases and insect pests are causing significant yield loss, and therefore, breeding for cultivars resistant to diseases and a pest is important. Majorly little millet crop is damaged by shoot fly (Upadhyaya *et al.*, 2015). Generally, small millets contain more nutritional properties than the major cereals, and huge variability exists in germplasm, as well as grain nutrients (Upadhyaya *et al.*, 2011). Exploiting existing variability present in germplasm and hybridization-derived variations could support breeding for the development of nutrient-dense cultivars along with the higher-yielding background.

In little millet, different breeding methods are used to develop the varieties, such as pure line selection, pedigree method, mass selection, and mutation breeding, which are valid to only self-pollinated crops. As of now, the little millet cultivars were released based on the selection from local landraces or cultivars, followed by hybridization and selection. However, in little millet hybridization process is difficult at the time of emasculation due to the small size of the florets; therefore creation of variability is complicated. Different emasculation and crossing methods are used for hybridization in little millet, such as the contact method, hand emasculation, hot water treatment, and USSR method, while these techniques have some disadvantages. Recently, a new emasculation technique was developed by GKVK, Bengaluru, India that is SMUASB (Small millets, University of Agricultural Sciences, Bengaluru) in 2014. In this process, cold water is used as a mechanical stimulator for the opening of florets and recorded a success rate of more than 50 per cent (Nandini *et al.*, 2019). Similarly, the utilization of hybrid vigor is restricted in small millets, due to difficulties in the hybridization programs. Thus, developing male sterile lines would be a possible selection to exploit heterosis, which is being effectively implemented in major crops for commercial hybrid seed production. So far, no reports recorded on male-sterile lines for hybrid development in little millet. However, mutation breeding has played a significant role in self-pollinated crops where hybridization is very difficult to create variability whereas two little millet varieties are released through mutation breeding in India.

However, the grain yield loss was recorded lowest in little millet (20%) compared to other minor millets under water stress and also recorded higher osmotic adjustment but did not result in higher productivity under water deficit (Ganapathy, 2017). Ajithkumar and Panneerselvam (2013) analysed the growth, chlorophyll pigments, osmotic adjustment and anti-oxidative enzymes activity in little millet under drought stress. Furthermore, stress treatment caused an increase in the activity of anti-oxidant enzymes such as superoxide dismutase, catalase and peroxidase. *Panicum sumatrense* possesses many growth and physiological drought tolerance characters which can be used in future breeding programmes. Bhaskaran and Paneerselvam (2013) investigated response and regulation of the antioxidant defense system and the level of lipid peroxidation under salt treatments. A great correlation exists between the anti-oxidant enzymes and lipid peroxidation. The defense mechanism activated in *Panicum* species studied was confirmed by the increased anti-oxidant enzyme activities under progressive NaCl stress. Although lipid peroxidation increased in *Panicum* species under salt stress the percentage of increase was low in *Panicum sumatrense*, indicating its salt- tolerant nature. Another possible conclusion is that improved tolerance to salt stress may be accomplished by increased capacity of the anti-oxidative system.

A number of little millet varieties have been released with high yield potential from different states. The list of little millet varieties developed through the above techniques is presented in Table 2 and some of the recent and popular varieties from different states are presented in Table 3.

Problems Related to Breeding

Varietal development through recombination breeding has received less attention for little millet compared to other small millet *viz.,* foxtail millet, and proso millet. However, the varieties were released and developed mostly through pure-line selection or mass selection. Breeding for improved varieties using hybridization techniques is inadequate due to small-sized florets and difficulties encountered in crossing (Nirmalakumari *et al.,* 2010). Detection of true F_1s requires for detection of morphological characters through molecular markers and is restricted to little millet. Little millet is considered one of the small millet species, and there required investigation, including the establishment of a genetic map and genome sequencing data for identifying the resistance genes for biotic and abiotic stresses. The potentiality of little millet has not been exploited in India due to the yield levels being very low and even cultivating the crop only in some tribal agricultural areas, but the nutritional status was higher compared to other cereals, therefore greater scope for exploitation of this millet under Indian condition.

Table 2 : List of little millet varieties released from 1989-2022

S. No	Variety	Pedigree	Location	Yield (Q/ha)	Year	Special features
1	Paiyur 1	Pure line selection	RRS, Paiyur TNAU	8-10	1989	Late maturity
2	BirsaGundli 1	Selection from Local	BAU, Ranchi	7 - 8	1993	Early maturity
3	TNAU 63	Selection from germplasm MS 2369	TNAU, Coimbatore	11 -12	1997	High grain and fodder yield
4	Paiyur 2	Pure line selection from accession PM 295	TNAU, Coimbatore	7. 5 - 8.5	2000	Late maturity
5	Kolab (OLM 36)	Mutant of SS -81 – 1	OUAT, Odisha	10-11	2001	Resistant to brown spot and sheath blight
6	Tarini (OLM 203)	Selection from local cultivar (KL 2) of Koraput Dist.	OUAT, Berhampur, Odisha	10-11	2001	Resistant to blast and grain smut
7	OLM 20	Mutant of SS-81– 1	OUAT, Odisha	11-12	2003	Drought tolerant
8	Co 4	Co 2 x MS 1684	TNAU, Coimbatore	16-20	2005	Non -lodging, suitable for double cropping
9	OLM 217	Selection from Udayagiri local	OUAT, Berhampur	15-16	2009	Resistant to rust and grain smut, but moderately resistant to sheath blight, tolerant to shoot fly
10	OLM 208	Selection from Lajigada local	OUAT, Berhampur	12-15	2009	Moderately resistant to shoot fly
11	JK 36	Selection from local Shahdolgermplasm	Rewa, JNKVV, Jabalpur	10-12	2009	Tolerant to shoot fly
12	JawaharKutki 4 (JK 4)	DLM 42 x Kutki 1	Rewa JNKVV Jabalpur	13-15	2016	Resistant to drought, lodging, and key pest Shoot fly and moderately resistant to head smut
13	PhuleEkadashi (KOPLM 83	Selection from local germplasm	ZARS, Kolhapur, MPKV rahuri	12-14	2016	Non-lodging.
14	GV-2	Derivative from mutant of released variety 'Gujarat Vari -1'	Waghai, NAU, Navsari	26-28	2016	Clean White color and bold seeded, Resistant to pests and diseases
15	Chhattisgarh Kutki2 (BL-4)	CO-2 x TNAU 97	Jagdalpur IGKVV, Raipur	10-12	2016	It has high iron content (28.3 mg/100 g grain). Tolerant to major pests
16	DHLM 36-3	Co-4 x Paiyur – 2	ARS, Hanumanmatti, UAS, Dharwad	14-16	2016	Late maturing variety
17	BL 6	Paiyur 1 x OLM 29	Jagdalpur IGKVV, Raipur	12-14	2016	Recommended for upland cultivation, and rich in zinc and calcium
18	ATL-1	Co-4 x TNAU 141	CEM, Athiyanthal, TNAU	15-16	2019	Duration: 110-115 days Drought tolerant and suitable for mechanical harvesting, Nutrient rich grains with high milling recovery. Kharif & Rabi The variety has been released and notified in 2019.The breeder seed is yet to be received from TNAU

19	CLMV 1 (JAICAR Sama 1):		IIMR, Hyderabad	18-20	2020	This variety can give a high tillering of 8-9 tillers. It is tolerant of insect pests and foliar diseases. It contains high protein (14.4%) and iron (58 ppm). It is recommended in the state of Maharashtra, Andhra Pradesh, Telangana, Tamil Nadu, and Puducherry.

Table 3 : Most recent and popular little millet varieties in cultivation

S. No.	State	Varieties
1.	Orissa	OLM 203, OLM 208, OLM-217
2.	Madhya Pradesh	JK-4, JK 8 and JK 36
3.	Andhra Pradesh	OLM 203, JK 8
4.	Tamil Nadu	Paiyur 2, TNAU 63 and CO 3,C0-4,K1, OLM 203,OLM 20
5.	Chhattisgarh	JK 8, BL 6, BL-4, JK 36
6.	Karnataka	OLM 203, JK 8
7.	Gujarat	GV 2, GV 1, OLM 203, JK 8
8	Maharashtra	Phule Ekadashi, JK 8, OLM 203

Genomic status

Genomic resources such as DNA markers, linkage maps, and genome sequences essential for gene tagging, QTL (quantitative trait loci) mapping, and marker-assistant selection are required to find the target genes that are responsible for the development of resistance with biotic and abiotic stress. Conventional breeding methods are used in describing the germplasm, and it is useful in the development and release of several cultivars with resistance to biotic and abiotic stresses. However, minor millets are acquiring lesser attention in public due to a lack of popular high-yielding varieties and proper mechanizations for seed processing. Recent reports on little millet have recorded less information about genome sequencing and transcriptome analysis, which is essential for creating new research programs (Akbar *et al.*, 2018). At present, crop improvement programs were extensively by using a molecular or marker-assisted breeding approach that utilizes molecular markers like Expressed sequence tags- simple sequence repeats (EST-SSR or Genic SSR) and genome-wide SSR markers with the use of widely genomic and transcriptomic resources available (Sandhu *et al.*, 2019). Transcriptome analysis mainly focused on the target genes and transcripts of thousands of genes in one place. (Desai *et al.*, 2021) sequenced the transcriptome of three samples, a total of 4443 genic-SSR motifs identified in 30,220 unigene sequences. These sequences are connected to gene expression regulation and response to environmental stresses. However, identified microsatellites could be used for future research for QTL-based breeding, genetic resource conservation, marker-assisted selection (MAS), and evolutionary genetics. Moreover, genomic marker-assisted development by utilizing various omics approaches can potentially enhance genetic gains in small millet improvement. However, a lack of molecular and genetic markers in little millet and no fine linkage maps and genome sequences appear to exist (Dwivedi *et al.*, 2012). Only limited information is available on the genetic diversity of little millet germplasm, whereas it is based on a limited number of DNA markers. A limited sequence information is available in the little millet crop that is needed for exploring the available markers from the maize, barnyard millet, and pearl millet as a result of the cereal millet synteny and also the availability of the SSR markers (Natesen *et al.*, 2020). The greater part of the information on genetic diversity analysis in little millet germplasm has used random amplified polymorphic DNA markers (RAPD) (Tiwari *et al.*, 2018) single nucleotide polymorphism (SNP), and genomic SSR markers. Further, DNA fingerprinting is the best alternative way to identify unique markers to differentiate the varieties. Natesen *et al.* (2020) had done the DNA fingerprinting analysis of five little millets genotypes such as BL6, ATL 1, TNPsu 176, Co (Samai) 4, Paiyur 2 using cereal SSR markers. The first time (Sebastin *et al.*, 2018) reported that the sequenced complete chloroplast (cp) genome of little millet is

139,384 bp in length with 38.6% overall GC content could be valuable information for the breeding programs of this Poaceae family of cereal crops.

Similarly, genetic transformation studies are useful for expressing the transgenes for resistance to different biotic and abiotic stresses, but in the case of little millet, none of the transgenes are available. However, immature embryos were used as explants for the transformation of genes derived from embryogenic callus. *In vitro* regeneration protocol was reported in little millet (Mishra, 2017) that could allow the transgenic work to be explored to introduce key genes to present resistance against various stresses. Detailed protocols for callus regeneration and transgene protocols have been available for all small millet species except little millet (Plaza-Wuthrich and Tadele, 2012).

Future Prospects

Little millet is an ideal crop for climate change and contingency plantings. It has more nutritional factors and lower glycemic index content suitable for more diabetic patients. Moreover, the storage capability of seeds is higher without any infestation of stored grain and pests. However, a lesser number of little millet germplasm available and lacking information on genetic diversity restricts the effective exploitation in crop improvement programs. Therefore, prioritizing germplasm collection is key to identifying trait-specific resources, genes, and alleles, which can be utilized in millet breeding programs. As of now, the little millet varieties were developed through mainly conventional breeding methods such as pedigree and mass selection, while no varieties from marker-assisted breeding. The yield level of the crop is very low compared to the other cereal grain crops. The yield level is very low in small millets and can be broken by a male sterility system and genomics-assisted crop improvement, together with better crop management and mechanization. But the above techniques are not fully developed in little millet. Genomic-assisted breeding will make easy the identification of new alleles and genes with better agronomic performance and resistance to biotic and abiotic stresses to speed up crop improvement. Biotechnology techniques such as tissue culture and genetic engineering are reported in related crops and could potentially support small millet improvement. The rapid development of sequencing technologies can generate millions of sequence reads at a low cost and in a short time, irrespective of whether there is prior sequence information or not. Additionally, public-private partnerships, public awareness, farmers' engagement across the countries who are interested in small millet research and promotion will be needed to incorporate small millet-based food products as an important source of nutrients in diets. Given the changing climate scenario and prevailing hidden hunger, greater research and developmental focus on little millet millets is the key to achieving food, feed, and nutrition security.

Conclusions

Among the small millets, little millet is one of the climate-resilient and nutrient-rich crops and it grows well under low-input agriculture. It is less affected by pests, and diseases and easily adapted to different environmental conditions, especially drought. The lack of high-yielding cultivars in the existing germplasm necessitates exploring the greater genetic diversity of germplasm. Moreover, the genome sequence of little millet is yet to be developed in little millet and lacks proper molecular markers for finding the targeted genes for resistance to biotic and abiotic stress. Nowadays, farmers are interested in millet cultivation because millets have more nutritional benefits, whereas it helps to tackle the malnutrition problems among billions of people. In recent times, research works are in progress to find the genetic diversity of little millet by using the genotyping-by-sequencing technique at ICRISAT, Hyderabad, and globally. Most research efforts should be on generating high-yielding varieties and processing while utilizing technologies. Germplasm resources combined with modern genomic tools which are help to accelerate the exploitation of genetic diversity in this crop.

Declaration

The authors declare that there is no conflict of interest.

Authors contribution statement

Venkata Ratnam Tata has written the manuscript and analyzed the data.

Madhavi Latha L has verified the data and corrected the manuscript.

Manoj Kumar D has also verified the data and supported in writing the article.

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