

Salinity Tolerance in Chickpea Genotypes during Germination

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ABSTRACT: A laboratory experiment for screening 25 chickpea genotypes collection from Zonal Agricultural Research Station (ZARS), Kalaburgi and from ICRISAT along with one susceptible (ICCV 96836) and one tolerant check (JG-14) for salinity tolerance was carried out at the Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur in a two factorial completely randomized design, with four replications. The first experiment consisted of two salinity levels viz., 0 mM NaCl (S₁) and 30 mM NaCl (S₂) as factor-I and 25 chickpea genotypes (G₁ to G₂₅) as factor-II. The second experiment was conducted to assess the performance (tolerance level) of five best identified genotypes from experiment I based on seed germination at higher salinity levels (up to 120 mM NaCl). The experimental results revealed that out of 25 screened genotypes in the first experiment, five genotypes, viz., BGD-103, ICCV-4958, WR-315, KCS-2 and KCS-4 recorded significantly higher seed germination (97.3, 98, 99.8, 98 and 99 per cent, respectively) and vigour (3115, 3220, 3555, 3061 and 3126, respectively) which were found tolerant to salinity stress at 30 mM NaCl stress level. These screened genotypes were further subjected for reconfirmation for their tolerance at 30 mM and also were checked for their performance under higher salinity levels (up to 120 mM) in the second experiment. The results revealed that with the increasing salinity levels, the seed germination and seedling vigour of all the chickpea genotypes decreased. Among the five genotypes, WR-315 recorded significantly higher seed germination (99.2 %) and seedling vigour index (2957) even up to 120 mM salinity level. Salinity adversely affects the seed germination and seedling establishment of chickpea. Among the 25 genotypes, BGD-103, ICCV-4958, WR-315, KCS-2 and KCS-4 were found tolerant upto 30 mM NaCl while the genotype, WR-315 was tolerant even at higher salinity level (120 mM NaCl). The study depicts that the genotypes BGD-103, ICCV-4958, WR-315, KCS-2 and KCS-4 can be used where the soil salinity is upto 30 mM NaCl while the genotype, WR-315 can be used where the salinity is up to 120 mM NaCl.

Keywords: Chickpea, Genotypes, Salinity, Screening, Seed germination, Tolerance

Chickpea (*Cicer arietinum* L.), a member of the family Fabaceae (Leguminosae), is an ancient cool season food legume. It is the only cultivated crop within the genus *Cicer*. Globally it is occupying an area of 14.56 million hectares with a production of 14.78 million tonnes which accounts for more than 20 per cent of the world pulse production. Much of the world chickpea supply (72 %) is from India and ranked first in area and production in the world, with an area of 10.56 million hectares, production of 11.17 million tonnes and productivity of 1077 kg ha⁻¹ [1].

Among the abiotic constraints, drought, chilling temperatures and soil salinity limit the productivity of chickpea [2]. Salinity is the concentration of dissolved mineral salts present in the soil (soil solution) and water.

Naturally salt-affected areas occur mainly in arid and semiarid regions [3]. Globally, about 1128 million hectares is affected by salinity and sodicity [4]. Salinity adversely reduces the overall productivity of plants by inducing numerous abnormal morphological, physiological and biochemical changes that cause delayed germination, high seedling mortality, poor crop stand, stunted growth and low yields. Salinity affects the availability of nutrients and water. Moreover, it induces osmotic stress; the physiological drought, which typically retards the growth and photosynthesis activity of plants and ultimately results in low yield [5].

Efficient strategies are required for better utilization of saline lands for crop production. Improvement of salinity tolerance in crop species is one of the potential strategies

in overcoming salinity problem in agriculture [6]. To explore the genotypic variability for salinity tolerance in chickpea, screening of the genotypes is very much necessary. Screening for salinity tolerance based on growth, yield and its attributes has become the method of choice by most of the labs worldwide. Recently seed physiological parameters have also gained recognition as important selection criteria for screening salinity tolerance in plants at early seedling stage due to the reliability of information attained. According to [7], chickpea is susceptible to salinity, especially during germination. Poor germination on saline soil results in poor establishment but some cultivars are affected more than others [8]. Hence, screening under laboratory condition is considered to be advantageous over field screening. Development of salt tolerant genotypes through conventional breeding programs is very slow due to the complexity of salt tolerance and lack of reliable traits for selection [6]. This hinders the development of accurate, rapid and reliable screening technique [9].

Salinity is the second most wide spread abiotic stress after drought which reduces crop productivity by impairing normal growth and metabolic processes and primarily causes high Na⁺ ion toxicity and osmotic stress and secondarily leads to oxidative stress and generation of Reactive Oxygen Species (ROS) that cause membrane damage and cell leakage and inhibition of photosynthetic efficiency, ultimately affecting growth and productivity [10]. It's been seen that over the years due to continuous and indiscriminate use of irrigation water even the good soils are getting converted to saline soil. Hence, in order to meet out the food requirement for the ever growing Indian population, efficient strategies are required for effective utilization of even these saline soils for crop cultivation. So, selection of a suitable genotype that can be grow even under saline soil is one of the strategies for effective utilisation of these saline soils. It is well known fact that a significant genotypic variation exists among the widely cultivated genotypes for salt tolerance. Further, the salinity level also differs considerably in different agro climatic zones. Hence, identification of suitable salt tolerant genotypes at different salinity levels is very much required to promote chickpea cultivation in saline soils. Hence, the experiment was planned to screen 25 chickpea genotypes based on seed germination and seedling growth in terms of root, shoot length and seedling vigour at seedling stage for salinity tolerance.

MATERIAL AND METHODS

The experiment was conducted at the Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur during 2019-20 in a two factorial completely randomized design with four replications. The seeds of the 25 genotypes were collected from Zonal Agricultural Research Station (ZARS), Kalaburgi, India. The first experiment consisted of two salinity levels viz., 0 mM NaCl (S₁) and 30 mM NaCl (S₂) as factor-I. Here, the germination papers were soaked in distilled water (control) and 30 mM NaCl solution, respectively for S₁ and S₂ and the germination test was carried out [11] for 25 chickpea genotypes (G₁ to G₂₅) as factor-II in order to screen them for salinity tolerance. The second experiment consisted of five salinity levels viz., 0 mM NaCl (S₁), 30 mM NaCl (S₂), 60 mM NaCl (S₃), 90 mM NaCl (S₄) and 120 mM NaCl (S₅) as factor-I. While, the factor-II consisted with the five best tolerant genotypes (G₁: BGD-103, G₂: ICCV-4958, G₃: WR-315, G₄: KCS-2 and G₅: KCS-4) which were identified as tolerant up to salinity stress at 30 mM NaCl stress level from the 25 chickpea genotypes used in experiment I based on seed germination. The different salinity levels were created as per treatments by dissolving known quantity of salt (NaCl) in known volume of water as per the table given below to get required salt concentrations, then the germination papers were soaked in the respective salt solutions and the germination test was carried out [11].

Sl. No.	Salt concentration prepared	NaCl dissolved / litre distilled water
1	30 mM	1.75 g
2	60 mM	3.50 g
3	90 mM	5.25 g
4	120 mM	7.01 g

Germination

The standard germination test was carried out by following between paper method [11]. Here, fifty seeds in four replications each were placed on the germination paper uniformly. The rolled towels were kept in the germination chamber maintained at 25 ± 2 °C temperature and 90 ± 5 per cent relative humidity using distilled water. Then the final count was taken on 8th day. The numbers of normal seedlings from each replication were counted and the mean germination was expressed in percentage.

Seedling growth parameters

At the time of germination, five normal seedlings were

selected at random from each replication and were used for measuring root and shoot length. The values were calculated and expressed in centimeter. The seedling vigour index-I was computed by following

Abdul-baki and Anderson, [12] and the mean values were expressed in whole number.

SVI-I = Germination (%) × Mean seedling length (cm).

Statistical analysis

The data collected from the experiments were analyzed statistically by following appropriate procedure [13]. Whenever F test was found significant, the critical difference (CD) values were calculated and the treatment means were compared at one per cent level probability ($p = 0.01$).

RESULTS AND DISCUSSION

Seed germination

Seed germination is usually the most critical stage in seedling establishment, which determines success of crop production [14]. Soil salinity is one of the important factors having a critical influence on seed germination, seed physiology and plant establishment [15]. The seed germination (%) was significantly influenced by salinity levels and genotypes. In the first experiment, a seed germination of 96.8 per cent was recorded under S_1 (control), while it was recorded as 94.5 percent under S_2 (30 mM NaCl) (Table 1). In comparison with control (S_1), there was 2.3 per cent (%) reduction in mean seed germination under saline condition (S_2 ; 30 mM NaCl). In

Table 1. Influence of salinity levels on seed germination, root length, shoot length and seedling vigour index-I of chickpea genotypes

Genotypes	Seed germination (%)			Root length (cm)			Shoot length (cm)			SVI-I		
	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean	S_1	S_2	Mean
G ₁ : ICCV 96836 (susceptible)	96.0	94.5	95.3	17.6	13.9	15.8	18.7	16.2	17.4	3488	2863	3162
G ₂ : JG-14(Tolerant)	100	99.5	99.8	18.1	15.0	16.5	21.6	20.1	20.8	3964	3492	3728
G ₃ : JG 11	87.0	85.0	86.0	16.9	15.4	16.1	18.2	17.5	17.9	3054	2795	2924
G ₄ : BGD-103	97.5	97.0	97.3	14.3	13.3	13.8	19.5	17.0	18.2	3296	2934	3115
G ₅ : GBM-2	97.5	96.0	96.8	15.9	14.6	15.3	20.2	18.2	19.2	3523	3151	3337
G ₆ : A1	97.0	95.5	96.3	16.9	15.2	16.1	18.3	16.7	17.5	3414	3044	3229
G ₇ : MABC-WR-SA1	98.5	95.0	96.8	16.2	14.5	15.3	20.1	17.5	18.8	3569	3084	3326
G ₈ : MABC-66-266	94.5	92.5	93.5	16.5	15.1	15.8	20.1	18.2	19.1	3462	3081	3272
G ₉ : WRC-411-111	97.5	94.5	96.0	18.3	13.3	15.8	18.4	16.7	17.5	3572	2838	3205
G ₁₀ : JAKI-9218	97.0	95.0	96.0	16.9	16.1	16.5	19.0	17.0	18.0	3481	3142	3311
G ₁₁ : NBeG-47	97.0	95.5	96.3	15.8	13.4	14.6	18.9	16.8	17.8	3365	2877	3121
G ₁₂ : NBeG-3	98.5	95.0	96.8	15.0	12.8	13.9	19.4	14.2	16.8	3395	2565	2980
G ₁₃ : ICCV-4958	99.0	97.0	98.0	15.5	14.5	15.0	19.7	15.9	17.8	3481	2959	3220
G ₁₄ : WR-315	100	99.5	99.8	19.7	16.4	18.0	18.2	17.0	17.6	3791	3320	3555
G ₁₅ : MNK-1	97.5	94.5	96.0	14.1	11.3	12.7	10.1	8.3	9.2	2353	1857	2105
G ₁₆ : MNK-Mutant-5-15	97.0	95.0	96.0	15.3	14.3	14.8	8.6	6.1	7.3	2317	1944	2130
G ₁₇ : KAK-2	93.0	91.0	92.0	13.2	10.5	11.8	9.9	8.0	9.0	2151	1684	1917
G ₁₈ : KCS-1	98.5	94.5	96.5	14.5	12.7	13.6	18.1	15.7	16.9	3212	2686	2949
G ₁₉ : KCS-2	98.5	97.5	98.0	16.3	15.2	15.7	16.6	14.4	15.5	3243	2879	3061
G ₂₀ : KCS-3	89.5	87.0	88.3	14.5	13.6	14.1	16.9	13.6	15.3	2818	2371	2595
G ₂₁ : KCS-4	99.5	98.5	99.0	15.0	13.1	14.1	18.9	16.1	17.5	3378	2875	3126
G ₂₂ : KCS-5	97.0	90.5	93.8	18.9	15.2	17.1	12.1	10.5	11.3	3008	2328	2668
G ₂₃ : KCS-6	97.5	96.5	97.0	16.4	14.6	15.5	15.8	13.2	14.5	3146	2694	2920
G ₂₄ : KCS-7	98.0	96.0	97.0	15.9	12.5	14.2	12.8	10.5	11.7	2627	2212	2419
G ₂₅ : KCS-8	96.0	91.0	93.5	15.1	12.5	13.8	11.5	9.8	10.6	2556	2029	2292
Mean	96.8	94.5	95.7	16.1	14.0	15.0	16.9	14.6	15.7	3186	2707	2947
S.Em. ±												
Salinity (S)		0.2			0.2			0.2			23	
Genotype (G)		0.7			0.5			0.5			82	
S×G		1.0			0.8			0.8			116	
C.D. @ 1%												
Salinity (S)		0.6			0.4			0.4			65	
Genotype (G)		2.0			1.5			1.5			229	
S×G		NS			NS			NS			NS	

Table 2. Seed germination and root length of the identified tolerant genotypes at higher salinity levels

Genotypes	Seed Germination (%)					Mean	Root length (cm)					Mean
	S ₁	S ₂	S ₃	S ₄	S ₅		S ₁	S ₂	S ₃	S ₄	S ₅	
G ₁ : BGD-103	98.0	97.0	96.5	95.5	94.0	96.2	14.7	14.1	13.0	11.1	9.4	12.5
G ₂ : ICCV-4958	99.0	97.5	97.0	96.0	94.5	96.8	16.5	16.0	15.8	15.0	13.3	15.3
G ₃ : WR-315	100.0	100.0	99.0	98.5	98.5	99.2	20.8	17.3	16.7	15.4	14.1	16.9
G ₄ : KCS-2	99.0	97.5	97.5	95.0	95.0	96.8	16.9	15.9	14.6	12.8	11.8	14.4
G ₅ : KCS-4	99.5	98.5	96.5	96.0	94.5	97.0	15.7	13.7	12.6	12.2	12.0	13.2
Mean	99.1	98.1	97.3	96.2	95.3		16.9	15.4	14.5	13.3	12.1	
		SE(m)±		CD@ 1%				SE(m)±		CD@ 1%		
Salinity (S)		0.4		1.2				0.4		1.2		
Genotype (G)		0.4		1.2				0.4		1.2		
S×G		0.9		NS				0.9		NS		

the second experiment (Table 2), the seed germination per cent showed concomitant decrease with an increase in salinity levels. The highest salinity level (S₅ - 120 mM NaCl) has recorded the lowest seed germination (95.3 per cent). The reduction of seed germination at high salt concentration might be due to osmotic stress [16]. Salinity might induce numerous effects on germination energy. Firstly; it reduces imbibition of water by lowering osmotic potential of the solution and thereby decreases the seed germination [17]. Secondly, it causes mineral imbalance and ion toxicity [18] which affects germination energy, germination capacity and germination percentage [19].

In the first experiment among the 25 genotypes, JG-14 (tolerant) and WR-315 showed significantly higher seed germination (99.8 per cent) while significantly lower seed germination (86.0 per cent) was recorded by JG-11 (Table

1). The chickpea genotypes which recorded lowest per cent reduction in germination were JG-14 (0.5 %), BGD-103 (0.5 %) and WR-315 (0.5 %). However, the highest reduction in germination was recorded under salinity level (S₂) was observed in the genotype, KCS-5 (6.7 %) and KCS-8 (5.2 %) (Fig. 1). In the second experiment, across five genotypes, WR-315 recorded significantly highest (Plate 1) seed germination (99.2 per cent), while the least seed germination (96.2 per cent) was recorded by BGD-103 (Table 2). These differences in the performance of chickpea cultivars may be due to genetic factors and heredity variation among the chickpea cultivars and also due to varied pattern of sodium uptake [20]. It was observed that under saline condition, the better performing genotypes had comparatively excess Na⁺ contents in all plant parts over other genotypes. This

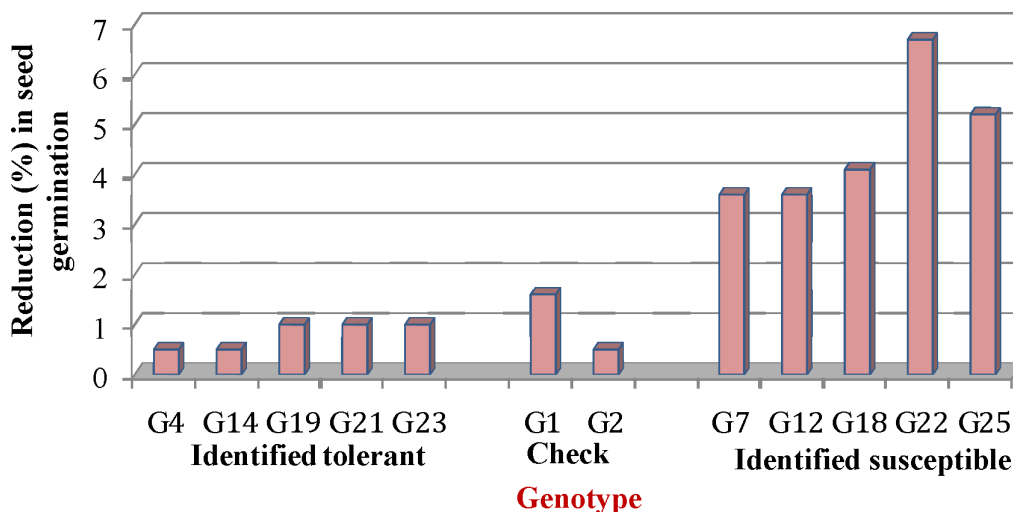


Fig. 1. Per cent reduction in seed germination at 30 mM NaCl (S₂) over control (S₁) of top five identified tolerant, two checks and five identified susceptible genotypes

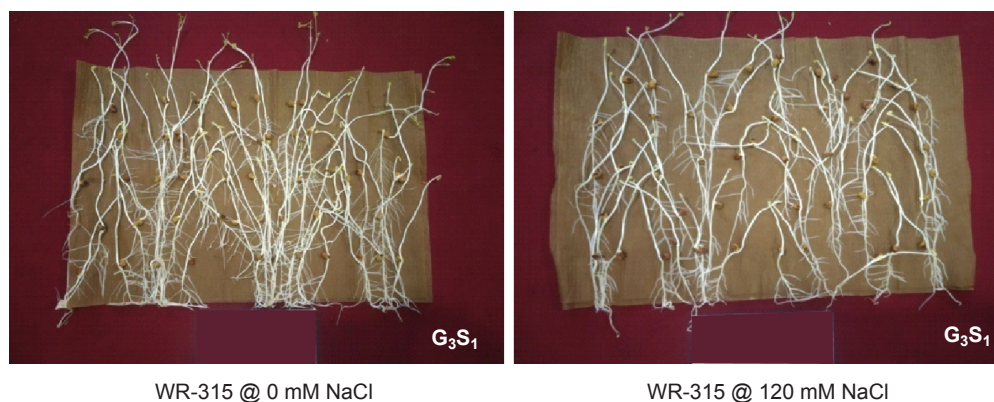


Plate 1. Genotype with highest seed germination at 120 mM NaCl (S_5) compared to control (S_1)

suggested that those genotypes might maintain their osmotic potential through accumulation of sodium in vacuoles. More accumulation of sugar is an account of high activity of amylase in tolerant genotypes. A greater accumulation of sugar lowers the osmotic potential of cells and reduces loss of turgidity in tolerant genotypes [21]. The sensitivity of plants to salinity depends on plant species and their developmental stage [21]. The decrease in germination under salt stress may be due to the fact that seeds seemingly develop an osmotically enforced dormancy under stress conditions. In both first and second experiment the data regarding the effect of interactions between salinity and genotypes for seed germination was found to be non-significant.

Seedling growth parameters

In the first experiment, the root length and shoot length and seedling vigour index-I was significantly influenced by the genotypes and salinity levels. Under saline condition (30 mM NaCl), significant reduction was observed in all the growth parameters including root

length, shoot length, and seedling vigour index-I. Under salinity (S_2 ; 30 mM NaCl) root length and shoot length were 14.0 cm (Table 2) and 14.6 cm (Table 3), respectively. Whereas in control, it was 16.1 cm and 16.9 cm, respectively. In the same way, under salinity (S_2 ; 30 mM NaCl) seedling vigour index-I was 2707, whereas in control it was 3186 (Table 3). In the second experiment, the root length, shoot length and seedling vigour index-I decreased with increase in salinity levels. The lowest values for root length (12.1 cm) (Table 2) and shoot length (7.4 cm) and seedling vigour index-I (1870) (Table 3) were recorded (Table 2) at highest salinity level, (S_5 - 120 mM NaCl).

Shaheenuzamn, [23] reported that salt stress reduced the seedling growth (root and shoot length) due to the inhibitory effect of ions. The reduction in root and shoot development may be due to toxic effects of Na^+ and Cl^- used as well as unbalanced nutrient uptake by the seedlings. These deleterious effects of salinity may result in a significant decrease in the photosynthesis and

Table 3. Shoot length and seedling vigour index-I of the identified tolerant genotypes at higher salinity levels

Genotypes	Shoot length (cm)						SVI-I					
	S_1	S_2	S_3	S_4	S_5	Mean	S_1	S_2	S_3	S_4	S_5	Mean
G_1 : BGD-103	14.7	14.1	13.0	11.1	9.4	12.5	3333	2998	1978	1738	1483	2306
G_2 : ICCV-4958	16.5	16.0	15.8	15.0	13.3	15.3	3518	3078	2571	2254	1978	2680
G_3 : WR-315	20.8	17.3	16.7	15.4	14.1	16.9	3900	3437	2776	2479	2196	2957
G_4 : KCS-2	16.9	15.9	14.6	12.8	11.8	14.4	3253	2857	2453	2070	1939	2514
G_5 : KCS-4	15.7	13.7	12.6	12.2	12.0	13.2	3362	2880	2049	1857	1755	2380
Mean	16.9	15.4	14.5	13.3	12.1		3473	3050	2365	2079	1870	
		SE(m)±		CD@ 1%				SE(m)±		CD@ 1%		
Salinity (S)		0.4		1.3				45		136		
Genotype (G)		0.4		1.3				45		136		
S×G		0.8		NS				100		NS		

increased respiration rate leading to shortage of assimilate to the developing organs, thus decreasing or completely stopping the growth [24]. Better seedling length as well as germination (%) was recorded in S₁ (control) as the toxic effect of sodium chloride was not there which ultimately resulted in higher seedling vigour index-I and better performance of the seedlings [25]. Seed vigour decreased with increasing salinity in Savory, [26].

In the first experiment among 25 genotypes, significantly higher root length (18 cm), shoot length (20.8 cm) and seedling vigour index-I were recorded by WR-315, JG-14 (tolerant) and JG-14 (3728) while the least root length (11.8 cm), shoot length (7.3 cm) and seedling vigour index-I (1917) was recorded by KAK-2, MNK-mutant-5-515 and KAK-2, respectively (Table 1). In the second experiment, across the five genotypes, significantly highest root length (16.9 cm) (Table 2), shoot length (12.9 cm) and seedling vigour index-I (2957) (Table 3) were reported in WR-315, while the least root length (12.5 cm), shoot length (11.2 cm) and seedling vigour index-I (2306) were registered in BGD-103, KCS-4 and BGD-103, respectively.

The difference in root length, shoot length and seedling vigour index-I among chickpea genotypes in response to salinity might be due to genetic factors [23-25]. The reduction in root length and shoot length for the salt sensitive genotypes might be due to more accumulation of Na⁺ which retards the cell division and elongation process and ultimately reduces the shoot length [27]. The reduced growth is a good indicator for sensitivity of genotypes to salt stress [28]. On the other hand the sensitive ones which had lower germination rate and seedling length, ultimately reported a lower seedling vigour index. Salt tolerance at seedling stage appears to be controlled by more than one gene and is highly influenced by salt concentration [29].

In the first experiment, the data regarding the effect of interactions between salinity and genotypes for root length, shoot length and seedling vigour index-I was non-significant. Whereas in second experiment, the interaction due to genotypes and salinity levels was found to be non-significant for root length and seedling vigour index-I, while it was significant for shoot length.

CONCLUSION

Based on the findings, it can be concluded that salinity adversely affects the seed germination and seedling establishment of chickpea. Among the 25 genotypes,

BGD-103, ICCV-4958, WR-315, KCS-2 and KCS-4 were found tolerant upto 30 mM NaCl while the genotype, WR-315 was found to be tolerant even at higher salinity level (120 mM NaCl).

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