Assessment of Kodo Millet Genotypes for Salt Stress Tolerance at the Seedling Stage Using Germination Tray

ABSTRACT

An experiment was conducted in a poly house setup using germination trays to investigate the effects of salt stress on the seedling growth and germination of 42 genotypes of Kodo Millet (*Paspalum scrobiculatum*). This research, utilizing a Completely Randomized Design with three replications, was conducted at the Research Farm of RVSKVV in Gwalior. To ensure uniformity and manage variability, a consistent mixture containing equal parts of compost, vermiculite, and cocopeat in a 1:1:1 ratio was used. Salt stress levels were evaluated using NaCl solutions of concentrations 50 mM, 100 mM, 250 mM, and 500 mM. Increased emergence time and a lower germination percentage were recorded at a 50 mM salt concentration compared to the control (0 mM). However, at higher concentrations, the effects were more severe, resulting in complete lethality with no recorded germination. At a 50 mM concentration, compared to the control, there was a decrease in shoot length, root length, seedling length, shoot fresh weight, root fresh weight, total fresh weight, total dry weight, vigour index, chlorophyll a, chlorophyll b and total chlorophyll concentration. Concurrently, an increase in proline concentration in the leaves was noted. The salt tolerance index and chlorophyll index was calculated to categorize genotypes based on their relative tolerance to salt-induced stress.

Keywords:kodo Millet, Salt Stress Tolerance, Salt Tolerance Index, Chlorophyll Index

1. INTRODUCTION

Kodo millet (*Paspalum scrobiculatum* L.) is an annual tetraploid cereal with a chromosome count of 2n = 4x = 40 and a genomic size of 1.91-1.98 Gb,originating from tropical Africa [1]. In 2023 world production of millets was 30,752 (1000 MT) led by by India with 40% of the global total. Top most millet producing states are Rajasthan (39%), Uttar Pradesh (17%), Gujarat (10%), Madhya Pradesh (10%) and Haryana (8%) [2]. For centuries, millets were staple crops in India. However, their prominence declined after the Green Revolution, which prioritized high-yielding varieties of wheat and rice [3].In order to encourage production and consumption of millets, Government of India notified millets as Nutri-Cereals in April, 2018. On proposal of India United Nations General Assembly (UNGA) declared 2023 as International Year of Millets on 5th March, 2021[4].

Kodomillet is generally cultivated in marginal environments and is resilient to most of the biotic and abiotic stresses. Millets present a promising solution for food security and nutrition due to their health benefits and low cultivation costs, making them a preferred crop. Additionally, they have the potential to provide biomass for bioenergy, which can reduce carbon emissions and promote sustainable agriculture. However, soil salinity is a significant abiotic stress affecting millet production in arid and semi-arid regions. Millets, classified as glycophytes, can only tolerate low levels of salt stress.Glycophytes experience severely stunted growth and may perish when subjected to salt concentrations of 100–200 mM [5] [6]. Therefore, developing salt-tolerant varieties of kodo millet is crucial. In India 17,10,673 hectare of land is salt affected [7].Climate change can further accelerate the process of soil salinization. The development of soil salinity in the root zone can occur due to several factors, including reduced water availability in arid and semi-arid irrigated agricultural regions, the upward movement of salts from shallow water tables, the reuse of

degraded waters, and saltwater intrusion. Most salinized soils are located in arid and semi-arid environments, characterized by low precipitation and high evaporation rates. [8]. It is observed that salinity negatively impacts the morphologyand physiology of millets [9].Reducing the entry of salt into the plant and decreasing the concentration of salt in the cytoplasm are the two primary mechanisms for achieving salt tolerance in plants[10]. Typically, the chlorophyll content in leaves decreases under salt stress [11]. Multiple researchers have discovered that salt-sensitive cultivars accumulate more proline under salt stress than salt-tolerant cultivars[12] [13] [14].It has been reported that salinity stress adversely affects seed germination and the overall growth parameters of seedlings [9] [15] [16]. Therefore, studying seedling traits and physiological parameters, such as proline and chlorophyll content, can provide insights into the salt tolerance capacity of different genotypes.Using salt-tolerant genotypes in agriculture offers several advantages. These genotypes can improve crop yield and maintain soil health by growing in saline soils without significant amendments, preserving soil structure and health over time. They reduce the need for fresh water and preserve genetic diversity, crucial for agricultural resilience against various stresses. Additionally, they support sustainable farming practices by utilizing marginal lands that are otherwise unproductive due to high salinity. Enhancing the salt tolerance of crops contributes to food security by expanding the range of arable land and ensuring a consistent food supply despite soil salinity issues. Thus, developing salt tolerant cultivars is extremely important.This study is primarily conducted to identify effect of NaCl induced salt stress on morphological and physiochemical traits ofkodo millet at the seedling stage.

2. MATERIAL AND METHODS

The experimental material comprises a total of 42 kodo millet genotypes, with 40 obtained from ICRISAT, Hyderabad, and 2 varieties, JK 137 and JK 155, sourced from Jawaharlal Nehru Krishi Vishwavidalaya, Jabalpur, MP, India. This research was conducted using a Completely Randomized Design with three replications for both control and stress conditions(50 mM, 100 mM, 250 mM, and 500 mM), took place at the Research Farm, Department of Genetics & Plant Breeding, College of Agriculture, RVSKVV, Gwalior, MP, India.The experiment was conducted in a poly house setup using germination trays. To standardize and control heterogeneity, a uniform mixture comprising equal parts of compost, vermiculite, and cocopeat in a 1:1:1 ratio was employed.Seeds were planted in germination tray with square cells of 4 cm height to approximately 1 cm of depth.In each cell four seeds were planted. Salt stress levels were assessed using NaCl concentrations of 50mM, 100mM, 250mM, and 500mM. A control group, irrigated with distill water, was maintained without salinity stress.For the stress condition, seedlings were irrigated with the desired NaCl solution concentration starting from the first irrigation itself. Observations on morpho-physiochemical traits were recorded 30 daysafter germination.

Ten morphological traits taken into consideration were days of emergence, germination percentage, shoot length, root length, seedling length, shoot fresh weight, root fresh weight, total fresh weight and total dry weight and vigour index. Physiochemical traits taken into consideration were proline content, chlorophyll a, chlorophyll b, total chlorophyll concentration, chlorophyll index and salt tolerance index. Proline and chlorophyll concentrations were measured following the methodologies of Sadasivam and Manickam [17] and Arnon 18], respectively. The mean data were used to estimate the analysis of variance and test of significance according to Burton method [19].

Determination of salt tolerance index

The salt tolerance index (STI) ofeach genotype is determined as a ratio of the total dry weight under salt treatment relative to the total dry weight under control condition.

$$\text{Salt tolerance index (STI)} = \frac{\text{Total dry weight under salt treatment}}{\text{Total dry weight under control treatment}}$$

Table 1:Scale for Salt Tolerant Index as given by Abdulrahman et al. [20]

Salt tolerance category	Range of salt tolerance index
Tolerant	80-100
Moderately tolerant	67-80
Moderately susceptible	54-66
Susceptible	Below 54

Determination of chlorophyll index

The level of change in total chlorophyll index (CI) due to salt stress was expressed as the ratio between total chlorophyll content in the stressed treatment and that in the control.

3. RESULTS AND DISCUSSION

At concentrations higher than 50 mM (i.e., 100mM, 250mM, and 500mM), the effects were severe, leading to complete lethality with no recorded germination.Therefore, all observations were recorded for control and salt stress conditions at a concentration of 50 mM NaCl.

The analysis of variance for 42 kodo millet genotypes was conducted for sixteen morpho-physiochemical traits. The mean sum of squares due to genotypes was highly significant for all morpho-physiochemical traits, except for germination percentage under control conditions (Table 2). Under 50 mM salt concentration, the mean sum of squares due to genotypes was highly significant for all morpho-physiochemical traits (Table 3).The significant mean sum of squares for all the genotypes under both normal and stress conditions indicates a substantial amount of

variability among the genotypes for the recorded traits. This variability can be harnessed in further breeding programs by selecting potential parents for different characteristics. Conversely, the non-significant mean sum of squares for germination percentage under control conditions suggests that the germination of all genotypes was almost uniform. Similar results were found by Kakar et al. [21].

Table 2. Analysis of variance for various morpho-physiochemical traits under control conditions

Source	Mean sum of squares														
	DF	DOE	GP	SL	RL	SGL	SFW	RFW	TFW	TDW	VI	Proline	Ch a	Ch b	Total Ch
Treatment	41	1.16**	126.91NS	8.31**	3.23**	18.18**	0.05**	0.03**	0.07**	0.06**	2047**	0.06**	2.84**	0.19**	3.08**
Error	84	0.27	143.84	0.01	0.03	0.05	0.01	0.02	0.02	0.09	2226	0.03	0.07	0.04	0.03

*ns P > 0.05; * P <= 0.05; ** P <= 0.01 DOE: days of emergence; GP: germination percentage; SL: shoot Length; RL: root Length; SGL: seedling Length; SFW: shoot fresh weight; RFW: root fresh weight; TFW: total fresh weight; TDW: total dry weight; VI: vigour Index; Ch a: chlorophyll a; Ch b: chlorophyll b; Total Ch: total chlorophyll*

Table 3. Analysis of variance for various morpho-physiochemical traits under salt stress conditions (50 Mm)

Source	Mean sum of squares																
	DF	DOE	GP	SL	RL	SGL	SFW	RFW	TFW	TDW	VI	Proline	Ch a	Ch b	Total Ch	CI	STI
Treatment	41	13.60**	1384.17**	10.18**	4.82**	27.19**	0.01**	0.03**	0.02**	0.09**	9924.02**	0.03**	1.50**	0.22**	2.22**	0.50**	0.17**
Error	84	0.27	228.17	0.02	0.02	0.12	0.07	0.05	0.04	0.03	13242.67	0.04	0.06	0.01	0.60	0.44	0.02

*ns P > 0.05; * P <= 0.05; ** P <= 0.01 DOE: days of emergence; GP: germination percentage; SL: shoot Length; RL: root Length; SGL: seedling Length; SFW: Shoot fresh weight; RFW: root fresh weight; TFW: total fresh weight; TDW: total dry weight; VI: vigour Index; Ch a: chlorophyll a; Ch b: chlorophyll b; Total Ch: total chlorophyll;;CI: Chlorophyll Index; STI: Salt Tolerance Index*

Morphological traits

Observations on root and shoot morphological traits were made 30 days after germination under both control and stress conditions.Under control conditions, the average number of days for emergence was 3.72, ranging from 3.00 to 5.33 days. The data reveal that the mean germination percentage of genotypes was 91.86%, with a range from 75.00% to 100.00%. The average shoot length was 7.78 cm, ranging from 4.56

to 11.10 cm. The average root length was 4.52 cm, with a variation from 1.63 cm to 7.10 cm. Seedling length varied from 7.73 cm to 17.30 cm, with a mean of 12.31 cm. The mean shoot fresh weight was 0.0307 g, ranging from 0.0113 g to 0.0517 g. Root fresh weight ranged from 0.0035 g to 0.0162 g, with an average of 0.0095 g. Total fresh weight varied from 0.0158 g to 0.0641 g, with an average of 0.0402 g. Total dry weight ranged from 0.0017 g to 0.0184 g, with an average of 0.0053 g. The mean vigour index was 1135.65, ranging from 643.33 to 1626.66 (Table 4).

Under 50 mM NaCl concentration, several parameters showed significant changes compared to the control condition. The number of days taken for emergence increased by 32.19%, with a mean emergence time of 4.92 days, ranging from 4.33 to 7.33 days. Germination percentage reduced by 55.72%, with an average of 40.67% and fluctuating between 25.00% to 83.33%.Six genotypes, namely Ips 181, Ips 287, Ips 319, Ips 694, Ips 785, and Ips 870, failed to germinate entirely.Mean shoot length decreased by 52.71%, averaging 3.68 cm and ranging from 1.83 cm to 6.43 cm. Root length decreased by 46.29%, ranging from 0.69 cm to 4.66 cm with an average of 2.42 cm. Mean seedling length went down by 50.54%, with an average of 6.08 cm, ranging from 3.66 cm to 11.10 cm. Mean shoot fresh weight showed a reduction of 63.51%, with an average of 0.0112 g and ranging from 0.0055 g to 0.0236 g. Root fresh weight decreased by 53.68%, averaging 0.0044 g and ranging from 0.0020 g to 0.0084 g. Total fresh weight decreased by 61.44%, with an average of 0.0155 g ranging from 0.0081 g to 0.0301 g. Mean total dry weight decreased by 54.71%, averaging 0.0024 g and ranging from 0.0007 g to 0.0091 g. Vigour index dropped by 74.47%, with a mean of 289.90 and ranging from 170.00 to 838.33(Table 4).

Delay in time taken for emergence and less germination percentage was recorded at 50mM salt concentration compared to control (0mM).However, at higher concentrations (100 mM, 250 mM, and 500 mM),it was determined to be entirely toxic, leading to a complete lack of seed germination compared to the control experiment.Similar results were also reported by Kothaiet al. [9],Alshiekheidet al. [16], Mushtaqet al. [22] and Prasanthi et al. [15].Under salt stress, one of the first physiological issues during seed germination is reduced water uptake due to the low water potential of the germination medium [23]. This leads to structural changes and metabolic disturbances, such as altered enzyme activities, disrupted nutrient mobility, nitrogen metabolism issues, imbalances in growth regulators, reduced hydrolysis and utilization of food reserves, and the accumulation of compatible osmotica like soluble sugars, free proline, and soluble proteins [24] [25]. These changes can cause poor or failed seed germination under saline conditions. Seed germination failure can cause significant losses, such as reduced crop yields, increased costs of replanting, labor, and other resources, and lower profitability. It can also delay crop maturity and harvest times, potentially missing optimal market windows. Unsuccessful crops can impact the local ecosystem, including soil organisms. These losses underscore the importance of ensuring optimal conditions for seed germination to maintain agricultural productivity and sustainability.

In saline conditions (50 mM), decreases were observed in shoot length, root length, seedling length, shoot weight, root weight, total fresh weight, total dry weight, and vigour index compared to the control. Analogous outcome were reported by Alshiekheidet al. [16], Mushtaq et al. [22], Hakim et al. [23] and Carpýcýet al. [26]. The primary response to the stress is the suppression of shoot and root growth. The initial decrease in shoot growth is likely due to hormonal signals produced by the roots.Salinity restrain root growth, limiting the soil volume accessible to roots and thereby reducing water and vital mineral uptake. This nutrient deficiency in the roots can lead to decreased growth in the shoot and overall decline in crop yield[27]. The small size of seedlings weakens them, reducing their ability to compete with weeds and withstand various biotic and abiotic stresses, which poses additional management challenges[28]. Inadequate root and shoot

development can also diminish the quality of harvested produce, affecting attributes like size, color, and taste. These factors ultimately lead to financial losses for farmers and impact profitability.

Table 4. Mean and range performance of germplasm entries under control and stress conditions

Traits	Mean		Reduction/ Increment(%)	Range			
				0mM		50 mM	
	0mM	50 mM	(Over control)	Minimum	Maximum	Minimum	Maximum
Days of emergence (days)	3.72	4.92	32.19	3.00	5.33	4.33	7.33
Germination percentage (%)	91.86	40.67	-55.72	75.00	100.00	25.00	83.33
Shoot length (cm)	7.78	3.68	-52.71	4.56	11.10	1.83	6.43
Root length (cm)	4.52	2.42	-46.29	1.63	7.10	0.69	4.66
Seedling length (cm)	12.31	6.08	-50.54	7.73	17.30	3.66	11.10
Shoot fresh weight (g)	0.0307	0.0112	-63.51	0.0113	0.0517	0.0055	0.0236
Root fresh weight (g)	0.0095	0.0044	-53.68	0.0035	0.0162	0.0020	0.0084
Total fresh weight (g)	0.0402	0.0155	-61.44	0.0158	0.0641	0.0081	0.0301
Total dry weight (g)	0.0053	0.0024	-54.71	0.0017	0.0184	0.0007	0.0091
Vigor index	1135.65	289.90	-74.47	643.33	1626.66	170.00	838.33
Proline (µg g−1 fresh weight)	0.1047	0.1835	75.26	0.0090	0.1900	0.0480	0.3860
Chlorophyll a (mg g−1 tissue fresh weight)	1.8439	1.2112	-34.31	0.3300	3.8300	0.5200	2.300

Chlorophyll b (mg g−1 tissue fresh weight)	0.4267	0.3870	-9.30	0.0700	0.9000	0.1200	0.9400
Total chlorophyll (mg g−1 tissue fresh weight)	2.2706	1.5982	-29.61	0.4200	4.6800	0.6700	3.1400
Chlorophyll index		0.7416				0.6250	0.9469
Salt tolerance index(%)		44.24%				18.67%	84.67%

Minus sign (-) indicates reduction in value of particular trait under salt stress condition compared to control

Physiochemical traits

The mean proline content was estimated at 0.1047 µg g−1 tissue fresh weight, ranging from 0.0090 µg g−1 tissue fresh weight to 0.1900 µg g−1 tissue fresh weight. The range of chlorophyll a content varied between 0.3300 mg g−1 tissue fresh weight and 3.8300 mg g−1 tissue fresh weight, with an average of 1.8439 mg g−1 tissue fresh weight across genotypes. Chlorophyll b content averaged 0.4267 mg g−1 tissue fresh weight, ranging from 0.0700 to 0.9000 mg g−1 tissue fresh weight. Total chlorophyll content ranged from 0.4200 to 4.6800 mg g−1 tissue fresh weight, with an average of 2.2706 mg g−1 tissue fresh weight.

Due to the lack of germination in six genotypes, physiochemical data is available for only 36 genotypes. When subjected to salt stress (50 mM), the mean proline content in genotypes increased by 75.26%, ranging from 0.0480 µg g−1 tissue fresh weight to 0.3860 µg g−1 tissue fresh weight, with an average of 0.1835 µg g−1 tissue fresh weight. Chlorophyll a content decreased by 34.31% under stress conditions compared to the control. The range of chlorophyll a content was 0.5200 mg g−1 tissue fresh weight to 2.300 mg g−1 tissue fresh weight, with an average content of 1.2112 mg g−1 tissue fresh weight across genotypes. Similarly, chlorophyll b exhibited a 9.30% decrease, with a mean of 0.3870 mg g−1 tissue fresh weight and a range from 0.1200 to 0.9400 mg g−1 tissue fresh weight. Total chlorophyll content declined by 29.61%, ranging from 0.6700 to 3.1400 mg g−1 tissue fresh weight, with a mean of 1.5982 mg g−1 tissue fresh weight.

The findings aligned with those reported by Alshiekheid et al. [16], Mushtaq et al. [22], and Sabir et al. [29]. Observations documented by Mir & Somasundaram [30] were consistent regarding proline content but differed concerning total chlorophyll content. It is considered that proline increases under salt stress condition due to its role as an osmoprotectant and a compatible solute as it helps in osmotic adjustment, acts as a scavenger of Reactive Oxygen Species (ROS), protecting cellular structures and biomolecules from oxidative damage, it can stabilize protein structures and membranes under stress conditions, thereby maintaining cellular integrity and function. Proline metabolism can regulate cellular redox potential, which is crucial for maintaining metabolic activities and reducing stress-induced damage [31]. The decrease in chlorophyll content under salt stress conditions can result from several reasons like disruptions in chlorophyll biosynthesis because salt stress interferes with the availability and uptake of essential nutrients like magnesium, oxidative stress-induced damage, ion imbalances, reduced CO_2 availability, and

cellular damage, collectively impairing photosynthetic efficiency and chloroplast function [32] [33]. Chlorophyll degradation directly impacts photosynthesis by reducing the plant's ability to capture light energy and convert it into chemical energy. This can lead to decreased plant growth and productivity.

Salt tolerance index (STI) and Chlorophyll Index (CI)

The salt tolerance index (STI) ranged from 18.67% to 84.67%, with an average of 44.24%. Based on this index, three genotypes (Ips 795, Ips 699, and Ips 176) were classified as tolerant, with an average STI score of group 83.00%. Five genotypes (Ips 4, Ips 741, Ips 777, Ips 358, and Ips 828) were classified as moderately tolerant, with a mean STI score of 71.66%. Eight genotypes were categorized as moderately susceptible, averaging an STI score of 60.18%, and the remaining genotypes were classified as susceptible, with an average STI score of 29.59%. The mean STI differed significantly across the different groups. These results are consistent with the findings of Kanawapee et al. [34]. Since the salt tolerance index (STI) here is based on total dry weight, it indicates the relative ability of different genotypes to maintain biomass production under saline conditions. This index allows for the comparison of genotypes performance by quantifying the impact of salinity on their total dry weight. Additionally, it aids in identifying genotypes with desirable traits for breeding programs aimed at developing salt-tolerant crop varieties.

The chlorophyll index (CI), varied from 0.6250 to 0.9469 with an average value of 0.7416. Genotypes with highest CI are Ips 814 (0.9469), Ips 627 (0.9459), Ips 699, Ips 176 and Ips 91. The chlorophyll index indicates the relative amount of chlorophyll present in plant leaves. It provides valuable insights into the photosynthetic efficiency and nutrient status of the plant, as nitrogen is the key component of chlorophyll [35]. It can help in early detection of stress in plants, allowing for timely interventions to mitigate the impact of stress.

4. CONCLUSION

Concentrations of salt exceeding 50mM are completely fatal for the plants. Under salt stress, there was a significant decrease in germination percentage, shoot length, root length, seedling length, fresh shoot weight, fresh root weight, total fresh weight, total dry weight, vigor index, chlorophyll a, chlorophyll b, and total chlorophyll concentration. Additionally, stress conditions caused a delay in seedling emergence. However, proline concentration increased under stress conditions, indicating a stress response mechanism. These results indicate that the period from sowing to seedling establishment is critically important for successful crop production.

Notably, genotypes Ips 699 and Ips 176 demonstrated tolerance to salt stress, not only based on the salt tolerance index but also due to their high chlorophyll index. Therefore, these genotypes, with higher salt tolerance index values based on total dry weight and high chlorophyll index values, can be instrumental in breeding plants that are more likely to thrive in saline environments. Use of tolerant genotypes against salt stress can offer several advantage in agricultural practices such as improved crop yield, maintain soil health as these genotypes can grow in saline soil without requiring significant soil amendments helping to maintain soil structure and health overtime, reduce the need for fresh water, preserve genetic diversity, which is crucial for the resilience of agricultural systems against various stresses, supports sustainable farming practices by making use of marginal lands that are otherwise unproductive due to high salinity. Enhancing the salt tolerance of crops contributes to food security by expanding the range of arable land and ensuring consistent food supply despite soil salinity issues.

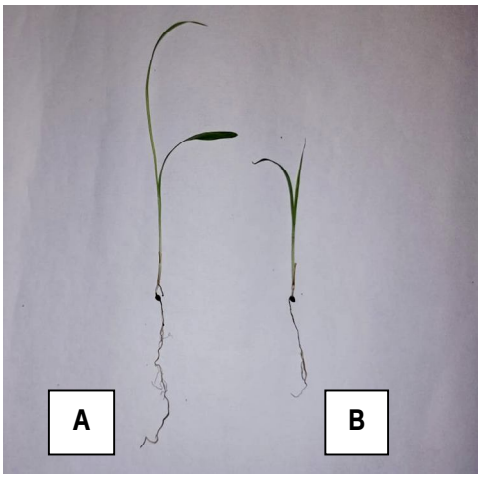

FIG.1.Difference between shoot length, root length and seedling length of seedling under [A] control (0 mM) and [B] salt stress (50 mM)

FIG.2.Screening of kodo millet genotypes for salt stress in germination tray

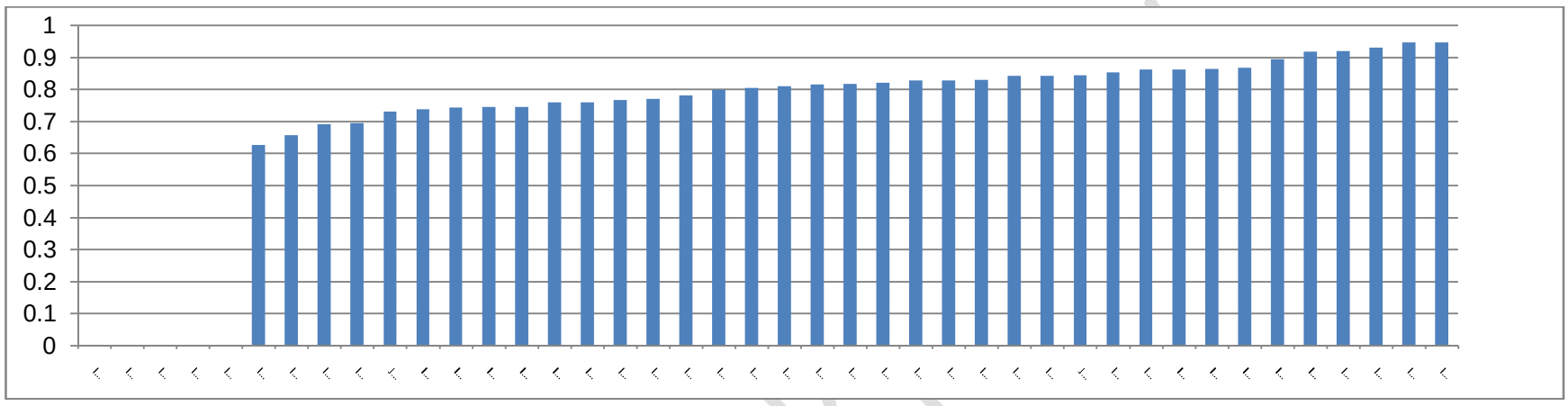

FIG.3.Variationin total chlorophyll index of kodo millet genotypes

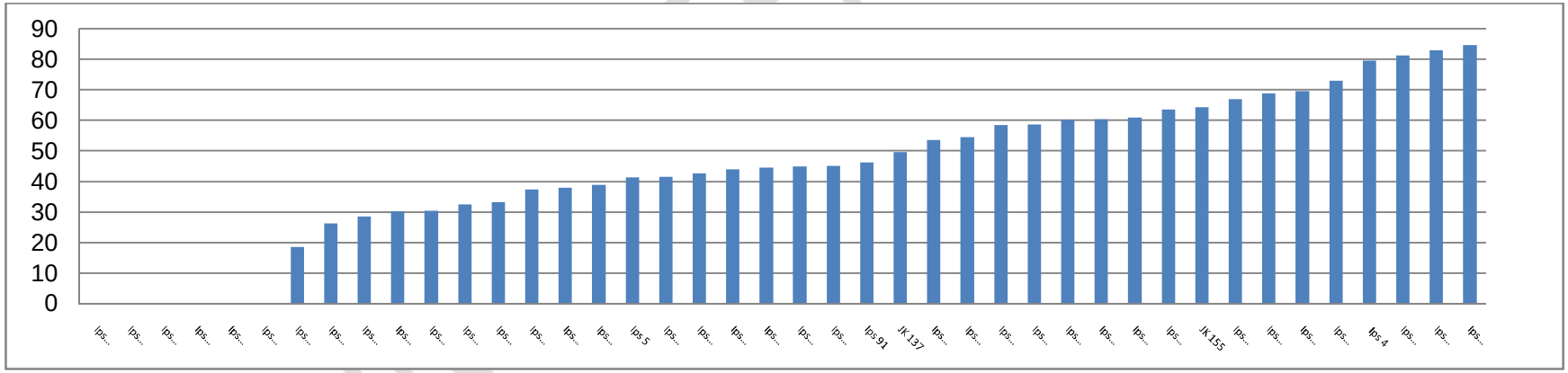

FIG.4. Variation in salt tolerace index of kodo millet genotyp

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