

Original Research Article

Qualitative analysis and statistical evaluation of curcumin content and total phenolic compounds in distinct turmeric (*Curcuma longa L.*) germplasms.

Abstract

Aim: Nowadays, turmeric value has increased with the presence of curcumin content and total phenols, as they are highly advantageous and useful in different areas of study. Salinity stress is also a major problem in certain areas under which many turmeric germplasms show reduced phenolic compounds and curcumin content. Therefore, the present study is aimed at identifying the different varieties that are diverse with high yields and maximum production so that the salt stress effects can be studied.

Methodology: This study was carried out using Factorial Completely Randomized Design (FCRD). Statistical analysis was done by applying the Duncan Multiple Range Test (DMRT) in relation to the concentrations of total phenolic compounds and curcumin content.

Results: We observed, a gradual rise in the total phenol content and curcumin content in all the germplasms. In particular, treatments including maximum salinity stress like 100 mM concentration gave better results compared to the control and 50 mM concentration (Fig. 2 & 3). These observations can be considered as stress-responsive mechanisms developed by the turmeric plants under salt stress conditions. Compared to all the selected germplasms, Vallabh Sharad and Selection 1 gave maximum output for total phenol content. 2.12 (leaf) and 0.28 (rhizome) values, which are maximum compared to controls observed in the case of 100 mM concentration. Also, Selection 1 gave 0.58 (rhizome) curcumin content, which is the maximum compared to the control.

Conclusions: Therefore, this increase in the phytochemicals in plants, helps in the increase of the medicinal properties in the turmeric plants also, internally protecting them from salt stress.

Key words: Total phenolic compounds, Curcumin content, Salt stress, Plant resistance.

Introduction:

Turmeric is an important medicinal herb which is commonly used as a spice and has several advantages in curing many important diseases like cancers, kidney ailments, and heart attacks. This spice has gained much importance in the ancient culture as it is used in treating many wounds, so it is commonly called as a wound dresser [1]. Also, in many places like China and Myanmar, turmeric powder is used as a paste for applying to inflammation and to other allergies. In India, turmeric paste is taken early in the morning to avoid many health issues and cleanse the gut. Not only this, turmeric is well known for its flavour, colour, and is also considered a super spice. Almost every Indian dish calls for a pinch of turmeric to add flavour, colour, and aroma. Turmeric is considered a natural colour for dyeing clothes and has many other advantages [2,3].

The presence of these unique compounds called phytochemicals in the rhizomes of turmeric makes this crop important and special [4]. These compounds promote several health benefits in humans, making this crop famous world-wide. This availability of total phenolic compounds and curcumin in the young and mature rhizomes also enhances the importance of this crop [5].

The levels of these phytochemicals increase as the rhizomes mature. These compounds are well-known constituents of plants with a role in protecting human health from severe cancers and promoting anti-oxidant activity. Along with this, the phytochemicals facilitate the elimination of the free radicals in the human body, which are highly responsible for causing cancers in humans. Such cancerous cells, that are formed will be eliminated with the presence of the total phenolic compounds in the turmeric rhizomes [6,7].

With respect to the many health benefits and medicinal properties in turmeric, which are internally achieved by the presence of the curcumin content in the rhizomes, this curcumin is the most abundant curcuminoid in the growing rhizomes of turmeric plants [8]. Of all the curcuminoids, curcumin occupies almost 85%, the others like dimethoxy curcumin have a total content of 15%, and bis-dimethoxy curcumin have a total content of 5%, indicating that out of total curcuminoids present in the rhizomes, curcumin stands out in its concentration in the young and maturing rhizomes of turmeric [9].

It is known that curcumin alone aids in curing many diseases like Cancers, Alzheimer's, Gall bladder stones and many others. Therefore, the presence and the content of the curcumin per rhizome also depends upon the variety of the turmeric plant selected [10]. The maximum rhizomes production with curcuminoids production indicates the high-quality turmeric which is useful in different industries like medicine, ayurvedic, homeopathy, drug preparations and nutritional experiments. So, proper understanding of the curcumin production and presence of total phenolic compounds in the turmeric rhizomes is an important factor in keeping many diseases at bay [11].

Recently, salinity stress in the turmeric plants growing, near water bodies or under improper field management practices has been observed to show reduction in yields as well as important compounds like curcuminoids and phenolic contents in both the leaves and rhizomes [12]. This problem of salt stress with respect to curcumin content and other important compounds, effects the quality of turmeric. Different plants exposed to different salt concentrations are known to develop varied symptoms like dried leaves, stunted growth, yellow patches on leaves etc., leading to the yield losses and reduction in phytochemicals. Therefore, our study is aimed at identifying the suitable cultivar that gives maximum phytochemicals under salt stress conditions so that it can be adopted and cultivated in the areas facing the problem of salinity. Along with this, the significance of these treatments is checked using FCRD (Factorial CRD) and the observations are analysed using DMRT (Duncan Multiple Range Test) [13].

Materials and Methods:

A total of five germplasms were selected for the work, which were known to have diverse backgrounds and beneficial characteristics like high yields and varied climatic adaptability. These germplasms were given salinity treatment in two concentrations; like 50 mM and 100mM. The germplasms were separated into control (untreated) and treatments (50 mM and 100 mM) groups. The sodium chloride solution mixed in required proportions (i.e., 2.92 g for 50mM and 5.48 for 100mM concentrations) with water was given to the plants and allowed to absorb the salt solution for two days without watering them [14]. The characteristics, growth and other parameters of the plants were observed and recorded at each and every month up to three months after the salt treatment to understand the effect of salinity on the plant's growth and phytochemicals. Three months after the salinity treatment, the rhizomes of

the plants were collected for observing and the estimation of total phenolic content and curcumin contents using spectrophotometer [14].

Phytochemical portray of different turmeric germplasms under salt stress conditions:



Figure 1: Stage 3 (3 months after salinity treatment) of germplasms selected for phytochemical analysis

All the five selected germplasms were estimated for their total phenolic content and curcumin content. All these germplasms were selected in the final Stage of the treatment, i.e., three months after salinity treatment. These germplasms comprise control, 50 mM concentration, and 100 mM salt concentrations of each cultivar. Their appearance, size, and growth varied with each other within the same cultivar due to different concentrations of salt. Also, some plants developed stress symptoms like leaf rolling, yellowing, light green patches on leaves, and reduced chlorophyll content, etc. These characters were seen only in treated germplasms of all the varieties, but the control plants were normal and showed normal growth conditions (**Figure 1**). Therefore, two plants of each cultivar, including both 50mM and 100mM, were taken for phytochemical analysis to understand the medicinal properties of turmeric germplasms even under salt stress conditions.

Extraction and Estimation of total phenolic compounds in turmeric germplasms under salt stress:

The total phenolic compounds in the turmeric plants that were salt treated, were estimated by using the standard protocol [15]. Here, the total phenolic compounds were estimated from the developing leaves and rhizomes of all the control and salt-treated plants. For this estimation, 0.5 gms of developing leaf and rhizome samples were taken and properly amalgamated with 10 ml of 80% ethanol using a mortar and pestle. To this amalgam the required reagents like Folin-Cio-Calteau's reagent was added to estimate total phenolic content and the samples were kept incubated in the 80% methanol overnight and estimated in case of curcumin content.

Spectrometric determination of total phenolics in turmeric germplasm:

Leaf and rhizome samples of 0.5g of all the plants were mixed with 8ml of double distilled water followed by adding Folin-Cio-Calteau's reagent (diluted in a 1:2 ratio with double distilled water). This reaction was incubated for 3 min at room temperature and immediately 1 ml of Na₂CO₃ (25% mixed in water) was added to halt the chemical reaction. This reaction mixture prepared was incubated for 60 min at room temperature, after which the absorbance was recorded at 725 nm. The blank value was recorded prior to the control and treatment values. These values differ for all the germplasms depending upon the control and the treatment concentrations.

Estimation of curcumin content:

In our study, young rhizomes (one month old) were selected for curcumin content estimation. Therefore, 1 gram of each rhizome was collected from the salt-treated and control plants from all the selected germplasms and were properly amalgamated using a few drops of methanol to which methanol was added further and the volume was made up to 100 ml and was kept overnight to infuse the curcumin present in the samples into the methanol. From the previously prepared solutions, a volume of 5 ml was pipetted out from them into fresh tubes, and the absorbance was recorded at 425 nm using a spectrophotometer. For the estimation of curcumin, the following formula was used, which was given by [15].

Curcumin content gm /100 gm =

$$\frac{0.0025 \times A_{425} \times \text{volume made up} \times \text{dilution factor} \times 100}{\text{weight of sample}}$$

$$0.42 \times \text{weight of the sample (gm)} \times 1000$$

The total mean values of both the phenols and curcumin contents were statistically represented using the Factorial CRD method, done using R-software [16] to check the significance of the data [17]. Also, with the help of Duncan's new Multiple Range Test, it was possible to provide the variations in the mean values of the germplasms taken and the treatments given that were significant to each other [18].

Results and Discussions:

The estimation of total phenolic compounds and curcumin content in turmeric germplasms under salt stress conditions was important to understand the effect of salinity on the growth and especially other parameters like curcumin content and total phenolic content [19,20]. Turmeric has gained much importance due to the presence of these two compounds called curcumin and total phenolics. It was well-known that total phenolics play a major role in treating many diseases in humans. Also, these total phenolics were known to act as very important anti-oxidants as they have the highest anti-oxidant properties [21].

Nevertheless, curcumin content was known for its famous role in protecting the human body against several cancers, heart failures, and kidney-liver dysfunctions. Apart from this, curcumin was known for its medicinal and therapeutic properties for wound healing and in the beauty industry [22,23]. The proper estimation of these important compounds of turmeric was useful in understanding the effect of salt stress on these varieties and thereby, cultivating these resistant varieties in the areas that were frequently affected by salinity [24,25].

This method of estimation and quantitative analysis of total phenolics and curcumin was possible by following certain methods which were previously designed [26,27]. For the estimation of these phytochemical compounds, not many chemicals were needed. Also, spectrophotometric evaluation and chromatographic techniques were known to be accurate in identifying and quantifying different phytochemical compounds [28,29].

Table 1. Phytochemical parameters under salt stress.

S. No	Turmeric germplasms	TOTAL PHENOLIC CONTENT (LEAF)			TPC (RHIZOME)		CURCUMIN CONTENT (RHIZOME)			
		Control	50mM	100 mM	Control	50mM	100m M	Control	50m M	100m M
1	VALLABH SHARAD	1.74	1.85	2.12	0.17	0.18	0.28	0.5	0.51	0.55
2	SELECTION 1	1.32	1.53	2.09	0.17	0.15	0.19	0.48	0.54	0.58
3	VALLABH PRIYA	1.06	1.46	1.87	0.16	0.15	0.19	0.47	0.51	0.51
4	AZAD	0.98	1.42	1.77	0.17	0.15	0.18	0.43	0.5	0.51
5	NEW SELECTION 2	0.56	1.21	1.36	0.15	0.12	0.16	0.41	0.42	0.42

The total phenolic content in both the leaves and rhizomes along with curcumin content were observed in the selected turmeric germplasms treated with salt stress (Table 1). Positive results were observed in all the treatments under salinity stress. It was observed that the 100mM concentration of all the germplasms gave maximum values i.e., 2.12, 2.09 for TPC (leaf) and 0.28, 0.19 for TPC (rhizome) followed by 0.55, 0.58 for curcumin content results compared to 50mM and control samples as a stress adaptive mechanism (Table1).

The total phenolic compounds present in the leaves and rhizomes, as well as the curcumin content in the rhizomes, were found to be normal i.e., 1.74 for TPC (leaf), 0.17 TPC

(rhizome) and 0.5 for curcumin content in control conditions, slightly increased in 50 mM concentrations, and maximum in 100 mM concentrations. Based on this experiment, out of all the five selected germplasms, Vallabh Sharad and Selection 1 gave good results i.e., 1.32, 1.53 and 2.09 for leaf and 0.17, 0.15 and 0.19 for rhizome samples compared to the other varieties for the total phenolic content.

In the case of total phenolic content in leaves, Vallabh Sharad gave normal results in control conditions and there was a slight increase in 50 mM concentration. But the value of 100 mM was maximum in a way that this cultivar was responsive to salt stress showed maximum values under treated conditions indicating the ability of the plants to cope with the stress (Table 1). The values observed in leaf samples were 1.74, 1.85, and 2.12 for control, 50 mM, and 100 mM concentrations, respectively. Following Vallabh Sharad, Selection 1 responded similarly with respect to the salt stress in which 1.32, 1.53 and 2.09 were observed for control, 50 mM and 100 mM concentrations. Similarly, total phenolic compounds in rhizomes were highest in Vallabh Sharad, with 0.17, 0.18, and 0.28 for control, 50 mM, and 100 mM concentrations, respectively. Also, New Selection1 showed similar patterns, i.e., 0.17, 0.15, and 0.19 for control, 50 mM, and 100 mM concentrations, respectively (Table 1). Here, in Selection 1 for rhizome samples, there was a slight reduction i.e., 0.17 in control sample to 0.15 in 50 mM treated samples for Total Phenolic Content and was greatly increased in 100 mM concentrations.

As a result, total phenol concentrations in control plants were normal in all varieties, slightly increased in 50 mM concentrations, and maximum in 100 mM salt concentration (Table 2). It was understood that under normal conditions the plants showed normal concentrations of total phenolic compounds, but in the case of 50 mM concentrations, the plants were undergoing stress tolerance mechanisms and were able to produce maximum phenolic compounds (Table 2). But in the case of 100 mM, it was clear that the plants were trying to cope with the stress of producing maximum phenolic compounds. This indicates that the increased salt concentrations in the samples improved the phytochemical accumulation in plants, i.e., increasing the total phenolic concentrations in a plant, which was one way of promoting improvement in growth of the plants through the increase in their phytochemical concentrations, which provide many useful effects in plant development and also health benefits in humans.

With respect to the curcumin content, which was estimated from the rhizomes of the plants that were three months old after the salinity treatment, normal results were obtained in control, a slight increase in 50 mM concentrations, and maximum values for both the phytochemicals was observed in 100 mM concentrations (Table 1). In Vallabh Sharad, where 0.50, 0.51 and 0.55 were observed for control, 50 mM concentration and 100 mM concentrations, respectively. Along with Vallabh Sharad, better results were observed in New Selection1 where 0.48, 0.54, and 0.58 concentrations were observed in control, 50 mM, and 100 mM concentrations, respectively (Table 1 &2, Figure 2). Therefore, it was observed that the curcumin content in untreated plants was less, compared to the treated plants like 50 mM concentration showing a little increase, followed by 100 mM concentration with a maximum increase. This was considered as a common phenomenon in which plants tend to develop certain characteristics against stress conditions and try to impose resistance. The increase in the curcumin content in 100 mM salt concentration increases anti-oxidant properties in plants, which helps humans in many ways.

Therefore, in terms of total phenolic content, Vallabh Sharad gave good results compared to New Selection1 in both the leaf and rhizome samples (Table 1&2). Whereas, Selection 1 gave the maximum results in curcumin content compared to Vallabh Sharad. In both the cases, there was a gradual increase in their phytochemical concentrations from control to 50 mM and 100 mM, which indicates that this increase in the total phenolic content and the curcumin content increases the ability of the plants to cope with the salt stress conditions, which in turn protects the crops from low yields and improves the quality of rhizomes.

Statistical Analysis:

With the help of Factorial CRD, the mean values for the germplasms along with the treatments were recorded in each phytochemical test. Here, DMRT helps in identifying those germplasms and treatments that were on par with each other and also certain germplasms and treatments showed significant variations among them [30].

Total phenolic content in leaf and rhizome:

The total phenolic compounds were considered as the most important phytochemicals in plants for their specific role in promoting growth under unfavorable or salt stress conditions.

Table 2. Total phenolic content along with DMRT in leaf and rhizome of selected turmeric germplasms under salt stress.

	TPC (LEAF)				TPC (RHIZOME)			
	CONTROL	50MM	100MM	MEAN	CONTROL	50MM	100MM	MEAN
VALLABH SHARAD	1.7	1.9	2.1	1.9 a	0.17	0.18	0.28	0.21 a
SELECTION 1	1.3	1.5	2.1	1.6 b	0.17	0.15	0.19	0.17 b
VALLABH PRIYA	1.1	1.5	1.9	1.5 c	0.16	0.15	0.19	0.17 b
AZAD	1	1.4	1.8	1.4 d	0.17	0.15	0.18	0.16 b
NEW SELECTION 2	0.6	1.2	1.4	1.0 e	0.15	0.12	0.16	0.14 c
MEAN	1.1 c	1.5 b	1.8 a		0.16 b	0.15 c	0.20 a	
CV				4.79				5.42

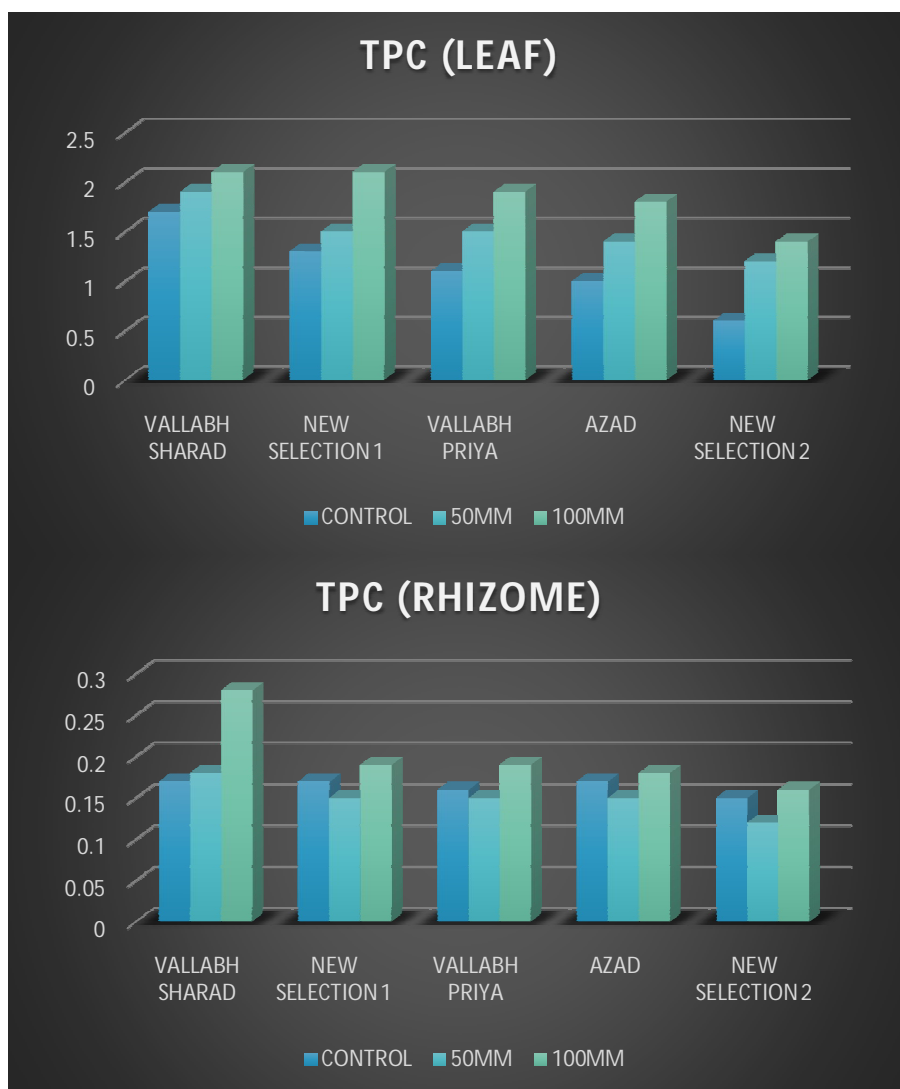


Figure 2: Graphical representation of total phenolic contents in leaf and rhizome samples

It was clear that the total phenolic content in the leaves was maximum in the case of Vallabh Sharad, i.e., 1.7, 1.9 and 2.1 in the case of control, 50 mM and 100 mM concentrations, followed by Selection 1, i.e., 1.3, 1.5 and 2.3 for control, 50 mM and 100 mM concentrations (Table 2). It was also observed that compared to control, plants in the 100mM treatment gave the maximum results as a mechanism of salinity stress tolerance, which was beneficial and advisable to cultivate these two varieties under stress conditions (Figure 2). Also, for DMRT, it was observed that all the three treatments and all the five germplasms showed significant differences among themselves; where the treatments were recorded as 1.1, 1.5 and 1.8 for control, 50 mM and 100 mM concentrations, followed by germplasms with their mean values

of 1.9, 1.6, 1.5, 1.4, and 1.0 respectively. Finally, the CV value of 4.79 indicates the significance of this salinity treatment.

The total phenols present in the rhizomes of the selected turmeric germplasms were maximum in Vallabh Sharad with the highest in 100mM concentration i.e., 0.17, 0.18, and 0.28 followed by New Selection1 with the highest in 100mM concentration i.e., 0.17, 0.15, and 0.19 for control 50mM and 100mM concentrations respectively (Table 2). In the case of DMRT, all the three treatments showed significant variation with each other, i.e., 0.16, 0.15, and 0.20 for control, 50mM and 100mM concentrations, and the germplasms Selection 1, Vallabh Priya, and Azad were observed to be on-par with each other with their mean values of 0.17, 0.17, and 0.16 respectively, leaving Vallabh Sharad and New Selection 2 as they showed significant variation, i.e., 0.21 and 0.14 compared to others (Figure 2). Finally, a CV value of 5.42 indicates the maximum significance of the treatment.

Curcumin Content:

Curcumin content was known to promote anti-cancerous and anti-diabetic properties along with many other health benefits. This curcumin helps in maintaining the human immune system due to the fact that its production was needed in maximum concentration to determine the quality of the plants (Table 3, Figure 3).

Table 3: Mean values along with DMRT of curcumin content

	CONTROL	50MM	100MM	
VALLABH SHARAD	0.5	0.51	0.55	0.52 a
SELECTION 1	0.48	0.54	0.58	0.54 a
VALLABH PRIYA	0.47	0.51	0.51	0.50 b
AZAD	0.43	0.5	0.51	0.48 b
NEW SELECTION 2	0.41	0.42	0.42	0.42 c
MEAN	0.46 c	0.50 b	0.52 a	
CV				5.21

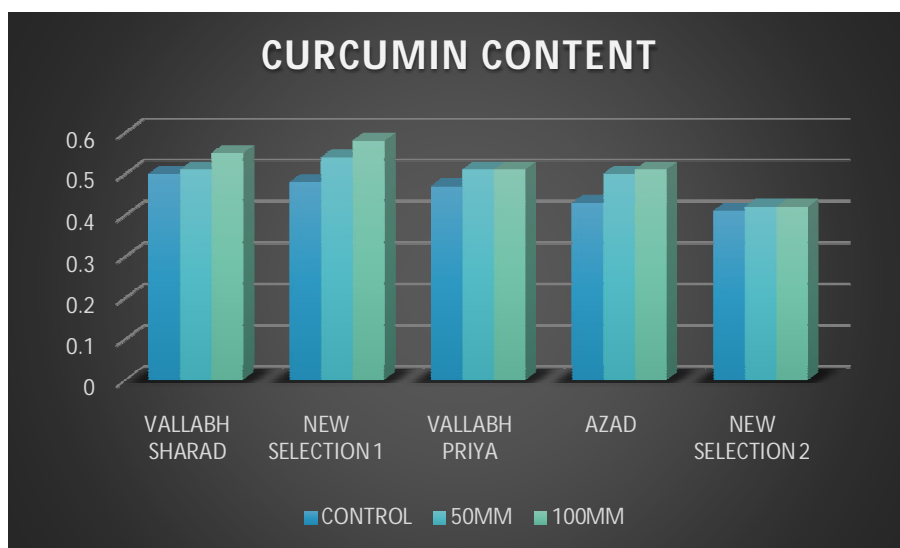


Figure 3: Graphical representation of curcumin content:

Curcumin content was typically highest during rhizome developmental Stages, as observed in our experiment, with New Selction1 having 0.48, 0.54, and 0.58 for control, 50 mM, and 100 mM concentrations, respectively, followed by Vallabh Sharad having 0.50, 0.5, and 0.55 for control, 50 mM, and 100 mM concentrations, respectively (Table 3). Also, the DMRT observed for curcumin content showed significant variation in all the treatments with their mean values of 0.746, 0.50, and 0.52 for control, 50 mM, and 100 mM treatments, respectively. In the case of germplasms, Vallabh Sharad and New Selection1 were on-par with each other with mean values of 0.52 and 0.54, respectively, followed by Vallabh Priya and Azad, i.e., 0.50 and 0.48, respectively, where New Selection2 was observed to be showing significant variation compared to other germplasms (Figure 3). Also, the CV of curcumin content, which was 5.21, shows maximum significance in the treatment.

Finally, these two varieties, Vallabh Sharad and New Selction1, gave maximum output for both the phytochemical parameters, i.e., total phenolic content and curcumin content, where it was very clear that Vallabh Sharad has the maximum total phenolic content in both the leaf and rhizome samples. Whereas, Selection 1 gave good results compared to Vallabh Sharad for curcumin content. Therefore, in the light of this information, it can be concluded and recommended for the use of these two germplasms, Vallabh Sharad and Selection 1, for cultivation in salt-affected soils to obtain maximum curcumin content in the growing rhizomes so that they will be beneficial to the farmers, industries, and consumers as well.

Conclusion:

The importance of turmeric was primarily due to the presence of total phenols and curcuminoids, which were collectively referred to as phytochemicals. Out of all the curcuminoids, curcumin occupies 85% of them, and so has many advantages for humans as well as for the plant itself. Total phenols are significant due to their anti-oxidant, anti-bacterial, and cardioprotective properties, among other benefits. These compounds protect human health and promote immunity. Curcumin has many advantages and is called as an active compound useful in treating cancers, heart diseases, kidney failures, Alzheimer's and many more. Both the compounds were highly significant for their anti-oxidant properties, which were useful in promoting health and immunity in humans. Not only this, curcumin under stress conditions protects the plants from death with its anti-oxidant properties, thus being beneficial to the plant itself.

In our study, we have observed that the total phenolic compounds and curcumin content vary in turmeric plants under salinity stress. Out of all the selected turmeric germplasms, only two gave good results compared to the other three with respect to the presence of these compounds under salt stress conditions. The samples were tested three months after the salinity treatment, at the time when the rhizomes were young or just emerged. Under such circumstances, it was understood that the concentrations of the total phenolic compounds were more in 50 mM treatment, followed by 100 mM, in both leaf and rhizome samples. Whereas, curcumin content was observed to be increasing gradually from control to 100 mM concentration and maximum content was observed at 100 mM concentration, which was a method of plant protection against stress conditions. Therefore, our studies reveal that some plants were able to tolerate salt stress conditions up to a maximum of 100 mM by scavenging the free radicals produced due to the stress and to increase the production of the phytochemicals as they provide many health benefits in humans due to their rich anti-oxidant nature.

Therefore, the importance of total phenols and curcumin along with its derivatives was needed for plant protection under severe salt stress conditions, and total phenolic compounds exclusively were important for plants and humans in promoting many health benefits, which were observed even under 100 mM salt concentration. Considering the above factors, Vallabh Sharad and Selection 1 germplasms gave maximum total phenolic compounds and curcumin content under salt stress conditions compared to their controls. Therefore, these two varieties

can be used and were highly recommendable in salt-stressed areas as they gave good phenolic and curcumin content even under severe salt stress conditions (100 mM) and were able to perform better than other selected turmeric germplasms under salinity stress influence.

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