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Evaluation of Different Extracting Methods of Inorganic Solutes (Na, K & Ca) Determination in Wheat for Salinity Screening Programs

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Abstract: A detailed study was conducted to compare different extraction/ digestion methods (Solute extraction by water (cold and hot), Solute extraction by weak acid (Acetic acid,CH_3COOH), Solute extraction by organic solvent (toluene), direct total analysis by wet oxidation (H_2SO_4, H_2O_2) and direct total analysis by wet oxidation ($HClO_4$: HNO_3) in 1:5 ratio), for determination of inorganic solutes especially (Na, K and Ca), to select most suitable, accurate efficient, and economical analytical methods for ionic analysis for smooth running of salt screening programs. Wheat crop was selected as test crop under saline and non-saline environments to observe the effect of genotypic and environmental variability on extracting methods. It was observed that there is wide variation in ionic contents among the extraction/ digestion methods. It was also observed that the determination of inorganic solutes especially (Na, K and Ca), by extracting method with 0.5% toluene water is highly correlated with wet digestion either by Nitric per chloric acid ($HClO_4$: HNO_3) in 1:5 ratio or sulphuric acid and hydrogen per oxide (H_2SO_4, H_2O_2). The extraction with 0.5% toluene water is safe, economical and efficient, therefore it is highly recommended to adopt the Solute extraction by organic solvent (toluene) method for salinity screening programs.

Keywords: Wheat, Salinity, Screening, inorganic solutes

1. INTRODUCTION

The tolerance of plant on sodium chloride is commonly, but not uniquely, related to the concentration of sodium in the shoot (Flowers, 2004). It has been reported that the plants adopt avoidance mechanism by restriction the higher uptake of Na ions in active parts (i.e. leaves), and accumulate in roots and stem. According to Ashraf and Leary (1995), salt tolerance is related to exclusion of ions in leaves from the all ages. The efflux of Na ions at the plasma membrane of root epidermis and cortical cells, and resorption of Na ions from xylem sap and its accumulation by xylem parenchyma cells are the processes involved in Na exclusion (Gorham *et al.* 1986). Sensitive cultivars accumulate Na ions more quickly than tolerant cultivars and this ion accumulation leads to leaf death and progressively death of the plant (Munns, 2002).

Studies on ionic relations in salinity screening programs are of prime importance. The role of ions in the adjustment of the osmotic potential of plants growing under saline conditions has been long established (Flowers, 1975). Regulation may start from selective absorption by the roots. The ions in the xylem sap may be re-absorbed by the xylem parenchyma cells before reaching the leaves or they may be in principal is re-translocated to growth medium. Within the shoot, preferential transport to old leaves leaving the younger actively growing leaves at an ion level at which photosynthetic activity could satisfy the requirements of growth.

However determination of solutes by suitable method is the main concern for efficient, accurate, less laborious and economical is necessary. Ansari 1982, while conducting work on salt tolerance in some grasses, concluded that dissolving in acetic acid or mere extraction in 100 mol m-3 acetic acid were equally good for detecting all the elements. As the extraction in 100 mol m-3 acetic acid is simple and quick, it can be used for analysis of solute. .The extraction methods usually require numerous steps which include pre-treatment of plant samples, extraction with some type of extracting solution and filtration before instrumental measurement. Increased complexity of the procedure can reflect on the accuracy of results and the cost of handling large numbers of samples.

At present different analytical methods are in use by different research organizations. Therefore the objectives of the present study are to compare different extracting/ digestion methods.

To select most suitable, accurate and efficient, analytical methods for cation extraction.

To evaluate the most economical methods for ionic analysis for smooth running of salt screening programs.

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2. METERIALS AND METHODS

For the evaluation of different extracting/ digestion methods wheat crop was selected as test crop. Four wheat genotypes were collected from two different sites (i.e. normal and saline (12 dSm^{-1}). The selection of wheat genotypes and salinity (i.e. saline and non-saline environment), was based on the evaluation of methods under wide variability of genotypes as well as wider variability of salt in the growing environment. Plants were harvested at maturity and chopped in small pieces and were dried in hot air oven for 72 hours / up to constant weight. The samples were grounded finely in grinding mill to pass (0.5) mm sieve. The homogenized samples were divided in to six groups for each site for extraction/ digestion. Extraction methods were Method 1: Solute extraction by water (cold), method 2: Solute extraction by water (Hot), method 3: Solute extraction by weak acid (Acetic acid ,CH_3COOH) (Ansari,1982), method 4: Solute extraction by organic solvent (toluene) (Weimberg *et al*., 1981), method 5: Direct total analysis by wet oxidation (H_2SO_4, H_2O_2) (Wolf 1982) and method 6: Direct total analysis by wet oxidation ($HClO_4$:HNO_3) in 1:5 ratio (Piper,1950)

The extracting methods were used for analyzing the Na, K and Ca from the plant material.

Total concentrations of cations were calculated by using the formula,

Na, K or Ca (%) in plant tissue = G.R x D.F./10000

Where:

G.R.= Graph reading

D.F.= Dilution factor

3. RESULTS

The analytical results of the cations (Na^+, K^+ and Ca^{2+}) extracted by different methods are individually described as under:

Sodium:

The data for sodium contents determined by different extracting/digestion methods is presented in **(Table 1)**. It was observed that sodium contents in wheat genotypes grown under saline site were higher as compared to non-saline site. Sodium contents plant samples from saline site were ranges from 0.79 to 3.73 %, whereas under normal soil conditions it ranged from 0.46 to 1.94 %, with mean values of 1.46 and 2.36 % under control and saline conditions, respectively. The data also showed that maximum sodium under two soil environments was observed in hot water extract (i.e. Method #2). The higher contents of Na in hot water in the present study were against the findings of Krejcova and Cernohorsky 2003), where, relatively low concentration of Na 0.01–0.02 mg. L^{-1} and K (0.1–100 mg. L^{-1}) in hot water extracts were observed.

The data was also analyze to observe the differences statistically, showed highly significant differences among the methods as well as among the salinity levels. The critical values for comparison (CVC) in case of extracting methods were (CVC = 0.1773) and among the salinity the CVC values were recorded as (CVC = 0.1024). Sodium values with respect to individual methods showed that there are five groups in which the means are not significantly different from one another. In this regard the most homogeneous group is Group #1. (Extraction by toluene and wet oxidation by H_2SO_4, H_2O_2) and Group # 2 (Acetic acid and wet oxidation by H_2SO_4, H_2O_2).

This was quite evident from the sodium values with respect to individual methods showing minimum variability between Method # 3, 4 and 5, under control condition (i.e. 1.70, 1.60 and 1.66%) and under saline conditions minimum variability was recorded in method of solute extraction by toluene and wet digestion by H_2SO_4, H_2O_2 (i.e. Method # 4 and 5 (i.e. 2.83 and 2.52 %, respectively).

Correlation studies to observe the existing relationships among the extracting/ digestion methods were also performed **(Table.2).** The studies were conducted on the basis of values observed under both growing conditions (control and saline). The data showed that there was a strong relationship among Toluene and wet digestion with HCl_2:HNO_3 showing values (r = 0.88), followed by extraction with distilled water and wet digestion with HCl_2:HNO_3 (r = 0.83) and wet digestion with HCl_2:$HNO_3$and wet oxidation by H2SO4, H_2O_2, (r = 0.82).

Potassium:

The data for potassium contents determined by different extracting/digestion methods is presented in **(Table 3).** The trend in case of potassium was reverse. Potassium contents in wheat genotypes grown under saline site were lower as compared to non-saline site. Potassium contents in plant samples from saline site were ranges from 0.27 to 1.67 %, whereas under normal soil conditions it ranged from 0.34 to 2.11 %, with mean values of 1.6 and 1.09 % under control and saline conditions, respectively. Potassium values with respect to individual methods showing minimum variability between Method # 2, 4 and 5 under control condition (i.e. 2.00, 1.99 and 2.00 %, respectively) and under saline conditions minimum variability was recorded in method of wet digestion with HCl_2:HNO_3 and wet oxidation by H_2SO_4, H_2O_2, (i.e. Method # 5 and 6) followed by method of solute extraction by toluene and solute extraction by hot distilled water (i.e. Method # 4 and 2 (i.e. 1.45 and 1.67 %, respectively).

The data was also analyze to observe the differences statistically, showed highly significant differences among the methods as well as among the salinity levels. The critical values for comparison

(CVC) in case of extracting methods were (CVC= 0.132) and among the salinity the CVC values were recorded as (CVC=0.076).Potassium values with respect to individual methods showed that there are four groups in which the means are not significantly different from one another. In this regard the most homogeneous group is Group #1. (Extraction with toluene and Extraction with hot distilled water) Group # 2 (Acetic acid and wet oxidation by H2SO4, H2O2).

Correlation studies to observe the existing relationships among the extracting/ digestion methods were also performed **(Table. 4).** The studies were conducted on the basis of values observed under both growing conditions (control and saline). The data showed that there was strong relationship among extraction with acetic acid and extraction with toluene, showing values (r =0.894), followed by extraction with H2SO4: H2O2 (r=0.886) wet digestion with HCl2: HNO3 (r=0.839) Higher relations Acetic acid extraction were also reported by Ansari and Flowers, 1986.

Calcium:

The data for calcium contents determined by different extracting / digestion methods is presented in Table 1. It was observed that Calcium contents in wheat genotypes grown under non-saline site were higher as compared to saline site. Calcium contents of plant samples from non-saline site were ranges from 0.27 to 1.61% whereas under saline soil conditions it ranged from 0.20 to 1.37%, with mean values of 0.87 and 1.25% under saline and non-saline conditions, respectively. The data also showed that maximum calcium under two soil environments was observed in hot water extract (Method 2).

The data was also analyze to observe the differences statistically, showed highly significant differences among the methods as well as among the salinity levels. The critical values for comparison (CVC) in case of extracting methods were 0.102 and among the salinity the CVC values were recorded as 0.056. Calcium values with respect to individual methods showed that there are six groups in which the means are not significantly different from one another .In this regard the most homogenous group is Group 1(Extraction by toluene and wet oxidation by H_2SO_4, H_2O_2).

This was quite evident from the calcium values with respect to individual methods showing minimum variability between method 4 and 3 under control condition (i.e1.59 and 1.61) and under saline conditions minimum variability was recorded in method of solute extraction by toluene and wet digestion by hot distal water means method 4 and 2 (1.25 and 1.37 respectively)

Correlation studies to observe the existing relationships among the extracting / digestion methods were also performed (Table.5).The studies were conducted on the basis of values observed under both growing conditions (control and saline).The data showed that there was an strong relationship among extraction with cold distil water and extraction with toluene showing values r =0.656, followed by extraction with acetic acid and extraction with toluene (r=0.634).

4. CONCLUSIONS

The studies related to the extraction/ digestion methods for determination of inorganic solutes especially (Na and K), indicates that the extracting method with0.5% toluene water is highly correlated with wet digestion either by Nitro perchloric acid ($HClO_4$: HNO_3) in 1:5 ratio or sulphuric acid and hydrogen per oxide (H_2SO_4, H_2O_2). In our opinion as the extraction with 0.5% toluene water is safe, economical and efficient, therefore it is highly recommended to adopt the Solute extraction by organic solvent (toluene) method for salinity screening programs.

Table 1: Na content in plants extracted / digested by different methods under control & Saline conditions

S. No	Methods	Control	Saline	Mean
1.	H_2O (cold)	0.46 i	0.79 h	0.63 E
2	H_20 (hot)	1.94 de	3.73 a	2.83 A
3	HOAc	1.71 ef	2.17 d	1.94 C
4	Toluene	1.60 fg	2.83 b	2.21B
5	H_2SO_4, H_2O_2	1.66 f	2.52 c	2.09 BC
6	$HClO_4$: HNO_3	1.37 g	2.14 d	1.75 D
Mean		1.46 B	2.36 A	---
LSD (0.05) for salinity				0.102
LSD (0.05) for Methods				0.177

Table.-2: Correlation between the amounts of Na extracted by different Extractants

Extractants	HCl 2:H NO3	H_2SO_4: H_2O_2	Toluene	Acetic acid	Distille Water (Hot)	Distilled Water (Cold)
HCl_2:HNO_3	-	-	-	-	-	-
H_2SO_4: H_2O_2	0.817***	-	-	-	-	-
Toluene	0.88***	0.65***	-	-	-	-
Acetic acid	0.70***	0.67***	0.551**	-	-	-
Distilled Water (Hot)	0.73***	0.75***	0.761***	0.531**	-	-
Distilled Water (cold)	0.83***	0.676***	0.778***	0.592**	0.823***	-

Table 3: Potassium (K) content in plants extracted/ digested by different methods under control & Saline conditions

S.#	Methods	Control	Saline	Mean
1.	H_2O (cold)	0.34 f	0.27 f	0.305 D
2	H_2O (hot)	2.00 a	1.67 b	1.83 A
3	HOAc	2.11 a	0.91 e	1.51 BC
4	Toluene	1.99 a	1.45 c	1.72 A
5	H_2SO_4, H_2O_2	2.00 a	1.13 d	1.565 B
6	$HClO_4$: HNO_3	1.13 b	1.09 de	1.11 C
	Mean	1.595 A	1.087 B	---
LSD (0.05) for salinity				0.076
LSD (0.05) for Methods				0.132

Table-4: Correlation between the amounts of K extracted by different Extractants

Extract ants	HCl_2: HNO_3	H_2SO_4 H_2O_2	Toluene	Acetic acid	Distilled Water (Hot)	Distilled Water (Cold)
HCl_2:HNO_3	----					
H_2SO_4: H_2O_2	0.828***					
Toluene	0.766***	**0.823*** **				
Acetic acid	**0.839*** **	**0.886*** **	**0.894*** **			
Distilled Water (Hot)	0.205	0.335	0.457**	0.267		
Distilled Water (Cold)	0.593***	0.510**	0.791***	0.594***	0.478**	

Table 5: Calcium (Ca) content in plants extracted/ digested by different methods under control & Saline conditions

S.No.	Methods	Control	Saline	Mean
1.	H_2O (cold)	0.27 f	0.20 f	0.23 D
2	H_2O (hot)	1.50 a	1.37 b	1.43 A
3	HOAc	1.61 a	0.61 e	1.11 BC
4	Toluene	1.59 a	1.25 c	1.42 A
5	H_2SO_4, H_2O_2	1.50 a	0.93 d	1.215 B
6	$HClO_4$: HNO_3	1.03 b	0.89 d e	0.96 C
Mean		1.25 A	0.87 B	
LSD (0.05) for salinity				0.056
LSD (0.05) for Methods				0.102

Table-6: Correlation between the amounts of Ca extracted by different Extractants

Extractants	HCl_2: HNO_3	H_2SO_4: H_2O_2	Toluene	Acetic Acid	Distilled Water (Hot)	Distilled Water (Cold)
HCl_2:HNO_3	-		-	-	-	-
H_2SO_4: H_2O_2	0.502**	-	-	-	-	-
Toluene	0.509**	0.590***	-	-	-	-
Acetic acid	**0.619*** **	0.491**	**0.634*** **	-	-	-
Distilled Water (Hot)	0.145	0.275	0.290	0.167	-	-
Distilled Water (Cold)	0.291	0.217	**0.656*** **	0.594***	**0.618*** **	-

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