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Molecular and Agronomical Assessment of Six Wheat (*Triticum aestivum* L.) Cultivars under Salt-stress Conditions

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ABSTRACT

The study was carried out during two seasons 2007/08 and 2008/09, respectively. It was conducted to determine the effect of salinity stress of four concentration levels on six wheat cultivars under green house conditions and to assess their genetic diversity by using RAPD technique. Split-plot design with four replications was used. The results showed no significant differences between salinity treatments for most of the characters under study except for plant height, number of tillers plant^{-1} and dry shoot weight in the first season and at plant height, number of leaves plant^{-1}, date to maturity, number of spike and grain weight/plant in second season. There were highly significant differences among varieties under salinity treatments for plant height and the number of tillers/plant in both seasons. No significant differences for interaction between varieties and salinity stress for both seasons. Six RAPD primers (OPA01, OPA03, OPA09, OPA13, OPA14 and OPA20) revealed polymorphism among the six wheat cultivars. RAPD markers were highly efficient and showed high variation among the six cultivars studied. The closer varieties genetically in the cluster behaved similarity in their response to salinity tolerance such as Candor and Debira which were genetically closely related as shown by the dendrogram and the second sisters Pohain and Wady Alnile.

Key words: Wheat cultivars, salinity stress, tolerance, growth, yield, RAPD

INTRODUCTION

Wheat (*Triticum asetivum* L.) is commonly classified as moderately salt tolerant (Khan *et al*., 2004) and for screening or developing salt tolerant wheat varieties, physiological and biochemical studies are necessary to identify the physiological and biochemical markers. By using these markers available wheat germplasm can be screened for salt tolerance or by incorporating them new high yielding salt tolerant wheat varieties can be developed. This is essential to fulfill the wheat grain yield demands of overgrowing (Khan *et al*., 2009).

Wheat has become an important stable food in Sudan. Being a temperate crop, it is not indigenous to Sudan; yet it was traditionally grown since early times in the Northern State (Lat. 18-22°N) that enjoys a relatively cooler and longer winter season than in Central Sudan (Lat. 18-22°N). However, owing to the increasing demand for wheat coupled with the limited resources in Northern Sudan, the expansion in wheat cultivation took place in warmer but comparatively less

expensive production systems of the central plains. Most of the salt-affected soils in the Sudan have a relatively low nutrient status and contain 0.01-0.02% organic nitrogen. The impact of salinity on agriculture is now being felt in irrigated areas in which soil and waterborne salts are accumulating during repeated cycles of water use. Non saline soils could easily be damaged and degraded by secondary salinization through irrigation with water from the Blue Nile, White Nile and River Nile (Elsheikh, 1998).

Randomly amplified polymorphic DNA sequences or RAPD markers are based on the amplification of unknown DNA sequences using single, short and random oligonucleotide primers (Ovesna *et al*., 2002). RAPD-PCR is currently used as genetic markers quite useful in breeding programs for assessment of genetic variability between genotypes (Hillel *et al*., 1992; Kahraman, 1999). RAPD molecular markers were used to study the genetic variability and relationships among wheat accessions (Cao *et al*., 1999), lines (Zhang *et al*., 2000), tetraploid wheat cultivars (Sivolap *et al*., 1997; Fahima *et al*., 1999; Szucs *et al*., 2000), wild and cultivated tetraploid wheat (Joshi and Nguyen, 1993), hexaploid cultivars (Mayburg *et al*., 1997; Labuschagne and Maartens, 1998; Sun *et al*., 1998; Cao *et al*., 2000).

Plants differ genetically in their response to salt stress (Ahmad *et al*., 2005). Different mechanisms of salt tolerance by plants have been suggested by different workers (Flowers and Hajibagheri, 2001; Gorham, 1994; Schachtman and Munns, 1992).

Keeping in view the importance of wheat and salinity, the present study has been planned to determine the effect of salt stress on Wheat cultivars under green house conditions and to assess their genetic diversity. Also, to identify presence of genotype specific markers using the Random Amplified Polymorphic DNA Technique (RAPD).

MATERIALS AND METHODS

The experiment was conducted in the glasshouse of Commission for Biotechnology and Genetic Engineering (CBGE), National Centre for Research, Khartoum, Sudan, under Saline conditions during the seasons 2007/08 and 2008/09, respectively; sufficient healthy seeds of each variety were sown in pot containing equal volumes of sand and clay (1:1) for 96 pots, divided into 4 replications of 6 cultivars namely: AL-Nilein, Candor, Pohain, Sasarib, Wady Alnile and Debira and 4 treatments (A = 50 mM NaCl, B = 100 mM NaCl, C = 200 mM NaCl and the control treatment Con=Zero). All pots were irrigated 17 weeks including 13 salt water as treatments. The data collected was subjected to Gomez and Gomez (1984) analysis of variance technique and LSD test at 5% probability level to compare the differences among treatments means (Steel and Torrie, 1984).

Using yield potential (YP) and stress yield (YS), the following quantitative criteria of drought tolerance were calculated:

- Tolerance index (TOL) and mean productivity (MP) (Rosielle and Hambling, 1981): TOL = YP-YS and MP= (YP+YS)/2
- Stress Susceptibility index (SSI) (Fischer and Maurer, 1978):

$$SSI= (1-YS/YP)/SI \text{ and } SI= (1- \acute{Y}S/\acute{Y}P)$$

  where, Si is stress intensity and ÝS and ÝP is mean of all genotypes in the stress and non stress conditions, respectively
- Geometric Mean Productivity (GMP) (Kristin *et al*., 1997; Fernandez, 1992): GMP = (YP) (YS)

Molecular investigation: Genomic DNA was extracted from fresh leaf tissues of the six Cultivars using modified CTAB method (Porebski *et al.*, 1997). The modification was made in intention to improve the quantity and the quality of the DNA. In this method the fine powdered plant materials were immediately transferred into 13 mL Falcon tubes containing 6 mL of pre-warmed lysis solution. Tubes containing the samples were then incubated in a water bath at 65°C with gentle shaking for 30 min and left to cool at room temperature for 5 min. Isoamylalcohol and chloroform mixture (1:24) was added to each tube and the phases were mixed gently for 5 min at room temperature to make a homogenous mixture. The cell debris was removed by centrifugation at 5000 rpm for 15 min and the resulted clear aqueous phases (containing DNA) were transferred to new sterile tubes. The step of the chloroform: isoamyl alcohol extraction was repeated twice. The nucleic acids in the aqueous phase were precipitated by adding equal volume of deep cooled isopropanol. The contents were mixed gently and collected by centrifugation at 4000 rpm for 10 min. The formed DNA pellet was washed twice with 70% ethanol and the ethanol was discarded after spinning with flash centrifugation. The remained ethanol was removed by leaving the pellet to dry at room temperature. The pellet was dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8) and stored at-20°C for further use. The extracted DNA samples were observed under UV illumination after staining with ethidium bromide and agarose gel electrophoresis. The purity and the concentrations of the DNA were then spectrophotometrically assessed following Sambrook *et al.* (1989) method.

RESULTS AND DISCUSSION

The analysis of variance (Table 1) revealed non significant differences between salinity treatments for most of the characters under study except plant height, number of tillers /plant and dry shoot weight at the first season and at plant height, number of leaves /plant, date to maturity, number of spike and grain weight /plant at second season. Moreover, there were highly significant differences among varieties under salinity treatments for plant height and the number of tillers/ plant in both seasons. On the other hand, there were no significant differences for interaction between varieties and salinity stress for both seasons (Table 1). These results indicated that most

Table 1: ANOVA table show the analysis of 6 cultivars effected by 4 treatment of Salinity on two seasons

	Source of variation							
	Season 2007/2008				Season 2008 /2009			
Results	Salinity (S)	Cultivars (C)	(S) x (C)	CV	Salinity (S)	Cultivars (C)	(S) x (C)	CV
Degree of freedom	3	5	15	n.d.	3	5	15	n.d.
Mean square								
Plant height (cm)	338.8*	236.9*	62.3 N.S.	25.8%	266.7*	596.3**	41.3 N.S.	18.2
No. of tiller	10.6**	1.8**	0.4 N.S.	94.6	1.3**	0.5 N.S.	0.3 N.S.	217.6
No. of leaves	60.3 N.S.	50.9 N.S.	51.9 N.S.	81.5	1.2 N.S.	5.4**	0.6 N.S.	13.5
Days to heading	77 N.S.	71 N.S.	44.5 N.S.	7.7	2530.2 N.S.	950.8 N.S.	790.5 N.S.	50.6
Days to maturity	398.6 N.S.	126.7 N.S.	129.2 N.S.	11.8	3734.5 N.S.	5538.2*	1771.1 N.S.	102.1
Dry shoot weight	5.9**	1.1 N.S.	0.4 N.S.	63.3	15.4 N.S.	13.6 N.S.	14.6 N.S.	103.2
No. of Grains/spike	30.7 N.S.	309.8*	72.2 N.S.	122.8	21.2 N.S.	47.9**	6.8 N.S.	103.3
Grains weight /spike	0.1 N.S.	0.2 N.S.	0.1 N.S.	116.3	0.01 N.S.	0.02**	0.00 N.S.	83.3
1000 grains weight	187.6 N.S.	1260.5*	295.4 N.S.	98.6	531.1 N.S.	1796.1 N.S.	2182.2 N.S.	255.9

*: Significant, **: Highly significant, N.S: Not significant, C.V: Coefficient of variation, L.S.D: Least Significant difference and n.d: no data

Table 2: The means of the growth and Yield parameters under the different level of salinity treatments for the two seasons 2007/08-2008/09

						LSD	
Source of means	Seasons	A	B	C	Con.	0.01	0.05
Plant height (cm)	2007/08	41.9	37.2	35.7	43.6	3.40	3.70
	2008/09	32.8	35.9	32.1	27.8		
Number of tiller	2007/08	0.6	0.6	0.1	1.7	0.30	0.30
	2008/09	0.2	0.1	0.1	0.5		
Number of leaves	2007/08	11.1	7.9	8.0	7.9	0.60	0.60
	2008/09	6.9	6.9	7.4	7.0		
Days to heading	2007/08	81.8	80.3	83.8	84.1	17.10	18.50
	2008/09	53.1	70.0	62.5	46.8		
Days to maturity	2007/08	81.1	86.5	81.2	89.4	24.40	26.60
	2008/09	29.8	55.6	48.1	32.2		
Dry shoot weight	2007/08	1.1	1.1	0.8	2.0	2.10	2.30
	2008/09	2.0	3.7	2.5	1.5		
Number of grains/spike	2007/08	9.3	8.9	6.8	9.0	1.50	1.60
	2008/09	2.0	3.7	2.5	1.5		
Grains weight/spike	2007/08	0.3	0.3	0.2	0.4	0.04	0.04
	2008/09	0.1	0.1	0.1	0.1		
1000 grains weight	2007/08	24.3	18.0	20.2	18.8	25.00	27.20
	2008/09	23.6	16.6	14.6	12.9		

Where: A = 50 mM NaCl, B = 100 mM NaCl, C = 200 mM NaCl, Con: No salt application and LSD: Least significant difference

of investigated traits were sensitive to salinity stress particularly the plant height and the number of tiller/plant similar results were reported by Aslam *et al.* (1989). The reduction in the value of these characters may be due to low uptake of water by plants as well as toxicity of Na and C1 because of their high concentration in the nutrient solution (Bhatti, 2004).

Salinity stress reduced greatly and significantly the value of the most investigated traits under study (Table 1). The reduction in the value of these characters might be due to the toxic effect of salt on plant growth (Bhatti, 2004).

The performances of varieties were variable according to the incidence of salinity (Table 2). The highest (0.4) grain yield under non-saline (Con) conditions was obtained by varieties AL-Nilein and Pohain, while the lowest (0.2) was produced by varieties Candor and Debira. When salinity stress was (A), the highest (0.3) grain yield was recorded for varieties Pohain and Wady Alnile and the lowest (0.1) for the varieties AL-Nilein and Debira (Table 4). When salinity stress was (B), the highest (0.3) grain yield was achieved by varieties Pohain and Wady Alnile and the lowest (0.1) by the varieties Candor and Debira. When salinity stress was (C), the highest (0.2) grain yield was achieved by varieties AL-Nilein , Candor, Pohain, Sasarib and Wady Alnile and the lowest (0.1) by the varieties Candor and Debira. some species being more sensitive than others to NaCl stress for wheat (Grant, 2003).Because different cultivars may adopt different mechanisms for tolerating high external salinity (Ali *et al.*, 2004; Gorham, 1994; Isla *et al.*, 1998). The way these compounds are accumulated differs between species and ranges from only one to several different compounds was being accumulated (Gobinathan *et al.*, 2009).

The varieties responded differentially to salinity stress for grain yield (kg ha^{-1}) as well as for the other vegetative traits. The highest value of salinity tolerance (YA/YCon = 1) was exhibited by varieties Candor and Wady Alnile. when salinity treatment was (A), while the most sensitive

(YA/YCon = 0.3) varieties to salinity at this treatment was AL-Nilein (Table 4). When the salinity treatment was (B), the most tolerant (YB/YCon = 1) variety was Wady Alnile and the most sensitive (YB/YCon = 0.5) varieties was AL-Nilein, Candor and Debira). When the salinity treatment was (C), the most tolerant (YC/YCon = 0.7) variety was Sasarib, Wady Alnile and the most sensitive (YC/YCon = 0.5) varieties AL-Nilein, Candor, Pohain and Debira.

Significant differences among the cultivars on number of grains/spike (Table 1). Where the highest (17.1 at first seasons and 5.6 at second) number of grains per spike was found in Pohain cultivar and the lowest number of grains per spike were found in AL-Nilein (Table 3). The highest 1000-Grains weight was found in Pohain (34.7 at first season) (Table 3) and the lowest weight was found in AL-Nilein (13.6 at first season) (Table 3). Significantly Pohain proved itself as the best among the other six cultivars in the yield production and the worth was AL-Nilein.

For the isolation of good quality DNA, a CTAB-based procedure was optimized in the present study that yielded high quality DNA free of phenols, which may inhibit the activity of Taq polymerase. Several primers were tested on the sex genotypes (*Triticum aestivum* L.). The primers

Table 3: The means of the growth and yield parameters among the different cultivars for the two seasons 2007/08-2008/09

								LSD	
Source of means	Seasons	N	K	P	S	W	D	0.01	0.05
Plant height (cm)	2007/08	35.6	37.6	38.6	40.5	46.8	38.6	4.10	4.50
	2008/09	24.8	24.3	39.1	35.1	34.2	35.4		
Number of tiller	2007/08	0.9	0.4	0.6	1.2	1.0	0.3	0.70	0.80
	2008/09	0.4	0.4	0.3	0.4	0.0	0.1		
Number of leaves	2007/08	7.8	8.1	12.3	8.0	8.1	7.8	0.60	0.70
	2008/09	7.1	7.4	6.4	7.5	7.6	6.3		
Days to heading	2007/08	83.8	84.3	82.2	84.6	79.3	80.8	20.70	22.60
	2008/09	56.1	49.8	53.0	55.9	71.3	62.5		
Days to maturity	2007/08	82.3	87.4	80.3	87.0	86.1	84.3	30.00	32.60
	2008/09	25.3	31.6	67.4	31.6	62.9	29.7		
Dry shoot weight	2007/08	1.5	1.4	1.0	1.2	1.5	1.0	2.60	2.90
	2008/09	1.4	1.8	5.6	1.3	3.4	1.3		
Number of grains/spike	2007/08	4.5	7.9	17.1	7.8	6.9	6.7	1.70	1.90
	2008/09	1.4	1.8	5.6	1.3	3.4	1.3		
Grains weight/spike	2007/08	0.3	0.2	0.5	0.3	0.4	0.2	0.05	0.05
	2008/09	0.0	0.1	0.1	0.1	0.1	0.1		
1000 grains weight	2007/08	13.6	14.6	34.7	17.8	27.9	13.7	30.60	33.30
	2008/09	8.9	6.7	24.1	9.6	18.5	33.8		

Where: N: AL-Nilein, K: Candor, P: Pohain, S: Sasarib, W: Wady Alnile, D: Debira, LSD: Least significant difference

Table 4: Salinity tolerance including Stress Susceptibility index (SSI) and Geometric Mean Productivity(GMP) for six Sudanese wheat genotypes

Varieties	Con	A	B	C	A/Con	B/Con	C/Con	GMP-A	GMP-B	GMP-C	SSI-A	SSI-B	SSI-C
N	0.4	0.1	0.2	0.2	0.3	0.5	0.5	0.2	0.3	0.3	0.8	0.5	0.5
K	0.2	0.2	0.1	0.1	1.0	0.5	0.5	0.2	0.1	0.1	0.0	0.5	0.5
P	0.4	0.3	0.3	0.2	0.8	0.8	0.5	0.3	0.3	0.3	0.3	0.3	0.5
S	0.3	0.2	0.2	0.2	0.7	0.7	0.7	0.2	0.2	0.2	0.3	0.3	0.3
W	0.3	0.3	0.3	0.2	1.0	1.0	0.7	0.3	0.3	0.2	0.0	0.0	0.3
D	0.2	0.1	0.1	0.1	0.5	0.5	0.5	0.1	0.1	0.1	0.5	0.5	0.5

Where: N: AL-Nilein, K: Candor, P: Pohain, S: Sasarib, W: Wady Alnile, D: Debira, A = 50 mM NaCl, B = 100 mM NaCl, C = 200 mM NaCl, Con: = No salt application, GMP: Geometric Mean Productivity, SSI: Stress susceptibility index

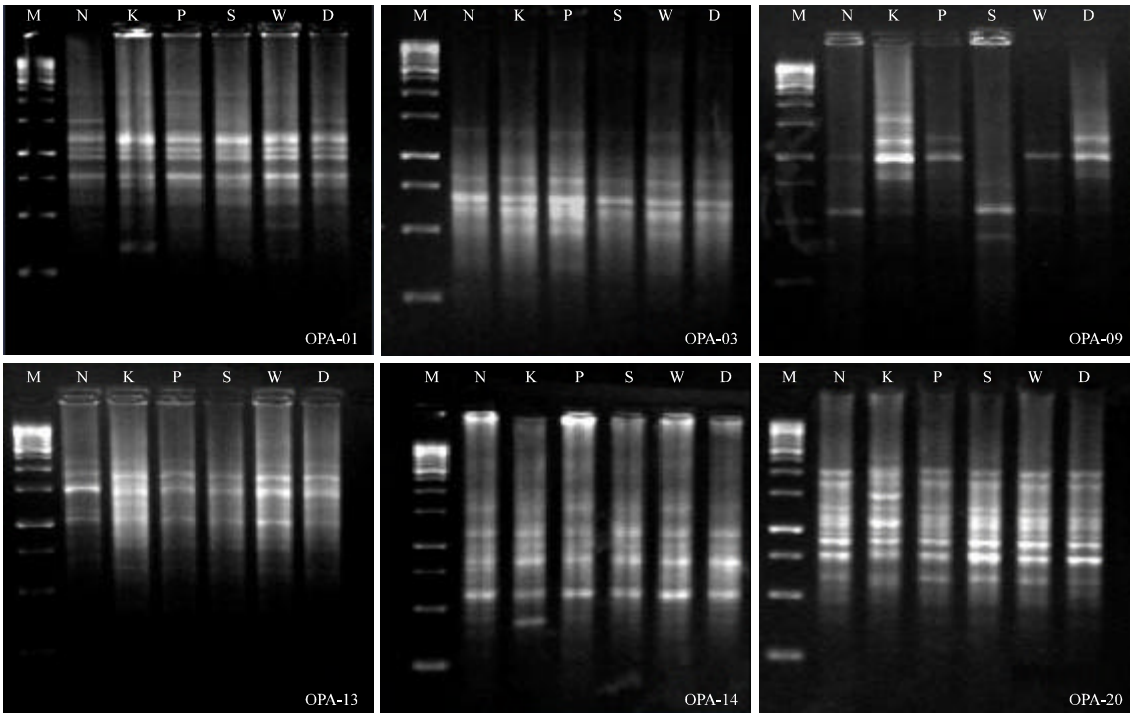

Fig. 1: RAPD amplification patterns with primers OPA-1, OPA-3, OPA-9, OPA-13, OPA-14 and OPA-20 (From left to right: lanes 1, 6 and M- ladder 1 Kb). N: AL-Nilein, K: Candor P: Pohain, S: Sasarib, W: Wady Alnile and D: Debira

Table 5: Polymorphism detected by the use of 6 random primers on the six wheat cultivars individuals (n.d. = no data)

Primer name	Sequence	Total No. of bands	No. of Polymorphic bands	No. of monomorphic bands	Percentage of polymorphic bands
OPA01	5'-CAGGCCCTTC-3'	9	6	3	66.7
OPA03	5'-AGTCAGCCAC-3'	7	4	3	57.1
OPA09	5'-GGGTAACGCC-3'	7	7	0	100.0
OPA13	5'-CAGCACCCAC-3'	10	8	2	80.0
OPA14	5'-TCTGTGCTGG-3'	7	4	3	57.1
OPA20	5'-GTTGCGATCC-3'	10	4	6	40.0
Total		50	33	17	400.9
Average		8.3	5.5	2.8	66.8

obtained from Operon technologies (Fig. 1). The result indicated that the six primers (66.8%) showed at least one consistent polymorphic band. The six informative primers were selected and used to evaluate the degree of polymorphism and genetic relationships among the genotypes under study. Total of 50 amplified fragments were distinguished across the selected primers and the statistical analysis showed 33 polymorphic bands among the genotypes with an average of 5.5 polymorphic bands per primer. The maximum numbers of fragment bands were produced by the primers OPA-13 and OPA-20 (10 bands) with 80% and 40% polymorphism, respectively while the minimum numbers of fragments were produced by the primers OPA-3, OPA-9 and OPA-14 (7 bands) with 57.1, 100 and 57.1% polymorphism, respectively. Pattern of RAPD fragments produced by the 6-mer primers OPA-1, OPA-3, OPA-9, OPA-13, OPA-14 and OPA-20 as shown in Table 5. As show in Fig. 2 there are two clusters. The first cluster (A) contained 4 cultivars AL-

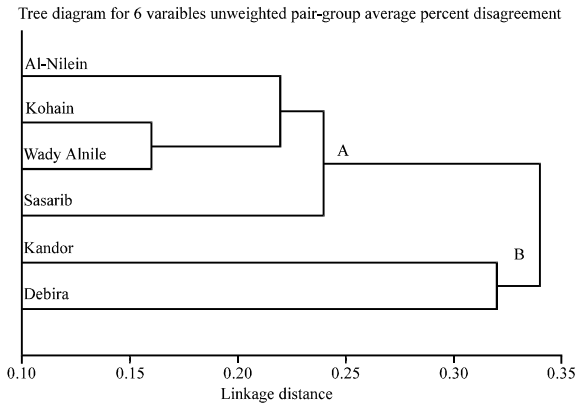

Fig. 2: Tree diagram constructed for 6 Wheat genotypes based on genetic distances using 6 RAPD primers

Nilein, Pohain, Wady Alnile and Sasarib. Where, Sasarib is an out-group for this cluster. AL-Nilein, Pohain and Wady Alnile fall in the same group. However Pohain and Wady Alnile are sisters and are more close genetically to each other than to AL-Nilein and Sasarib. In the second cluster (B) Candor and Debira have the same genetic distance and seems to be close genetically.

The molecular analysis used in the present study provides a simple means for the verification of phylogenic relationships and identification of wheat genotypes, which characterized by high yield over all favorable and stressful environments, will facilitate the breeding programs for wheat improvement. Liu *et al.* (1999) suggested that, based on RAPD markers, it is possible to differentiate wheat lines with different performances and that the classification of parents from these markers is of predictive value for developing superior lines. Several investigators used RAPD molecular markers to study the genetic variability and relationships among accessions, lines and cultivars of wheat (Taghian and Abo-Elwafa, 2003).

Candor and Debira were the lowest Salinity tolerance of grain yield under non-saline (Con), the second treatment 100 mM NaCl (B) and the third treatment 200 mM NaCl. They were the most sensitive under stress yield of the second treatment (YB/YCon = 0.5) and under stress yield of the third treatment (YC/YCon = 0.5) (Table 4). This similarity was clear in the Tree Diagram (Fig. 2) which it show Candor and Debira as a sister.

Pohain and Wady Alnile were the highest Salinity tolerance of grain yield under the first treatment 50 mM NaCl (A), the second treatment 100 mM NaCl (B) and the third treatment 200 mM NaCl beside AL-Nilein and Sasarib (Table 4). In the Tree Diagram (Fig. 2) show Pohain and Wady Alnile as a sister and in the same time AL-Nilein and Sasarib were sharing Pohain and Wady Alnile in the same cluster.

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