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The Development of Distinctive Multi-cut a New Alfalfa Variety "Naimat or GR-722" Performs Well in Salt-affected Soils

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ALFALFA (*Medicago sativa* L.) is a perennial legume fodder crop which known as a queen of fodders due to its valuable characteristics. It provides green fodder during lean periods of fodder scarcity. Alfalfa is moderately tolerant to salinity stress, so breeders mainly focus to develop salt tolerant varieties. A naimat (GR-722) variety was developed by the breeders from the random selection of five lines through the mass selection breeding method. The variety was evaluated in different experiments with check variety during 10 years of study. The variety was tested in different fodder yield trails along with check variety (Sargodha Lucerne). The performance alfalfa variety (Naimat) excellent in all fodder yield trials as compared to check and advance lines of alfalfa. Naimat variety also performs well in salt-affected soils as compared to other varieties. This variety is high green fodder yielding, tolerant to salinity has also a wide range of adaptability. It benefits the fodder growers, especially on salt-affected soils.

Keywords: Alfalfa (*Medicago sativa* L.), Fodder, High yielding, Salt tolerance.

Introduction

Alfalfa or Lucerne *Medicago sativa* L. (2n= 32, Family Leguminosae) originated in South West Asia is well adapted to a wide range of climatic and different soil conditions (Annicchiarico, 2015). It grows extremely well in dry climates on fertile soils where there is plenty of moisture available. It is the oldest recorded crop grown for forage. Due to its deep tap root system, it is tolerant to drought and very tolerant to heat. Alfalfa produces a large quantity of seed under hot dry climatic conditions. It is the highest in feeding value of all commonly grown forage crops. It is important legume in the world which is grown on more than 30 million hectares (Annicchiarico, 2015).

Alfalfa is a multicut perennial legume fodder crop. It is known as the queen of fodders, because of its distinctive and multiple characteristics. It provides green fodder throughout the year especially during the two periods of fodder scarcity in the province, May- June and October– November. It has long been considered an important source of vitamin "A". These characteristics make alfalfa a desirable ration components of farm animals. Horse farmers look to alfalfa as an excellent green fodder, hay and pasture crop and is being grown on large scale near big cities of the Punjab province. It also adds nitrogen to soil and is an excellent soil improver. It is being grown for green fodder, hay and silage for centuries.

Different environmental stresses affected on the life cycle of plants, soil salinity is one of these stresses which limits the growth and yield of plants (Forestan et al., 2016). Salinity mainly interferes the plant growth because it imposes two stresses on plants i.e. hyperosmotic pressure which results from the accumulation of salts and availability of low water and ion toxicity (Na+ ion) (Volkov et al., 2004). Soil salinity reduces the plant growth and lowers the crop yield in worldwide (Zhu et al.,

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2015). The world land about 930 million hectares is affected by soil salinity and it is expected that more land will become affected by salinity due to some reasons i.e. global warming, irrigation of land with salt-affected water, low precipitation and evaporation of soil moisture (Rokebul et. al., 2017).

Therefore, breeders are mainly focused to develop salt tolerance varieties of Alfalfa to minimize yield losses, especially in salt-affected areas (Bertrand et al., 2015). Alfalfa is moderately tolerant to salinity (Bertrand et al., 2015) and among legume crops; it has highly salt tolerant mechanisms (Munns & Tester, 2008). There are huge variations in germplasm for the salinity tolerance among the alfalfa genotypes which are identified and screen out that are the best source for plant breeders to develop a variety/advance line that has a tolerance to salinity (Long et al., 2015; Zhang et al., 2016; Benabderrahim et al., 2020).

So, the development of cultivars that are adapted in stressful environments would require a significant effort in the breeding and selection of alfalfa. The study of diverse germplasm and the generation of genetic parameters for different agronomic and yield traits would be desirable and beneficial for alfalfa breeders. Alfalfa is a perennial, outcrossing legume crop, so the improvement in yield and other yield contributing traits is highly compromised by tetrasomic inheritance, long selection cycles, highly inbreeding depression and presence of genotype and environment interaction for these complex traits (Bingham et. al., 1994; Brummer et. al., 1999; Annicchiarico et. al., 2010). The recurrent phenotypic selection is mostly used in alfalfa breeding programs to select genotypes with improved yield and tolerance to biotic/abiotic stresses in challenging environments while implementation of new breeding methods and approaches cannot be ignored (Li & Brummer, 2012; Annicchiarico et al., 2015; Biazzi et al., 2017).

Salinity has serious socio-economic repercussions on the agriculture production system of Pakistan. Almost 6.67mha area of Pakistan is affected by salinity and sodicity, while most of the soil is saline-sodic in nature. According to an estimate nearly 40000ha of land are being lost due to salinity and water-logging every year in Pakistan. The biological approach is one of the best ways to utilize the salt-affected soils where there is no water available and soils are sodic in nature. Alfalfa may be produced efficiently under saline conditions if farmers adopt salt-tolerant varieties and recommended salinity mitigation practices for its cultivation. It is fact that the non-availability of salt-tolerant high fodder yielding alfalfa varieties is the main hurdle in the way to enhancing forage production in the Pakistan. To cope with these problems, Fodder Research Institute in Sargodha launched an extensive research/breeding program on the alfalfa plant. The main objective of this research program is to develop high fodder-yielding alfalfa varieties for salt-affected areas. Therefore, in continuation of these efforts, Fodder Research Institute, Sargodha has developed a new Alfalfa variety "Naimat (GR-722)" which has high green fodder yield and is also moderately tolerant to salinity. Its production on salt-affected soils will be beneficial for fodder growers.

Materials and Methods

Study region

The Sargodha region is part of the agriculture area with low precipitation and mostly wheat is cultivated in this area. This region showed diverse climatic conditions. A GR-722 "Naimat" was compared with other varieties in field experiments during eleven consecutive years. All experiments (except zonal, national experiments) were conducted at the research farm of Fodder Research Institute, Sargodha, Pakistan.

Breeding Method

The hybridization work for the development of the alfalfa variety "Naimat (or GR-722)" was started during the year 2007 at Fodder Research Institute Sargodha. Initially, a heterogeneous base population was developed by random mating of five different salt-tolerant elite lines I-Con-B, 5-IN-59, No.1103, GR-800 and No.53. The resultant enriched base population was planted on an area of 35 x 50 m^2 in isolation and allowed to undergo open pollination for three years to attain maximum genetic variability. Mass selection method with five selection cycles was applied to the basal population to develop this variety. In each cycle, superior plants having higher forage yield, plant height, leaf area, number of leaves/tiller, resistance to diseases were selected and their seed was bulked. Hamd Alla et al. (2012) reported that

mass selection in genetically diverse populations is an effective method for the development of high fodder-yielding alfalfa varieties (Fig.1). Zhang et al. (2007) also have demonstrated that the success of selection programs for improvement of forage yield and its components in alfalfa is mainly dependent on the genetic variation existing within the initial selection population. After obtaining a uniform type, the candidate variety was tested in station yield trials during Rabi 2014-15 and 2015-16. Simultaneously, it was also evaluated under zonal trial during Rabi 2016-17 to test its performance for green fodder yield in different ecological zones of Punjab. Further, it was evaluated in National Uniform Fodder Yield Trials (NUFYT) during Rabi 2017-18 and 2018-19 throughout the country. Randomized Complete block design (RCBD) with three replications was followed in station, zonal and national uniform fodder yield trials. Each plot consisted of 4 rows with 6 meters in length. Green fodder yield data per plot was recorded in all yield trials. Qamar et al. (2000) also adopted the same procedure for the evaluation of alfalfa genotypes for green fodder yield. The seed of promising line 'GR-722 along with check variety 'Sargodha Lucerne' was supplied to the Deputy Director Federal Seed Certification and Registration Department, Sargodha to conduct "DUS" trials and recording of plant characteristics during the years 2017-18 and 2018-19.

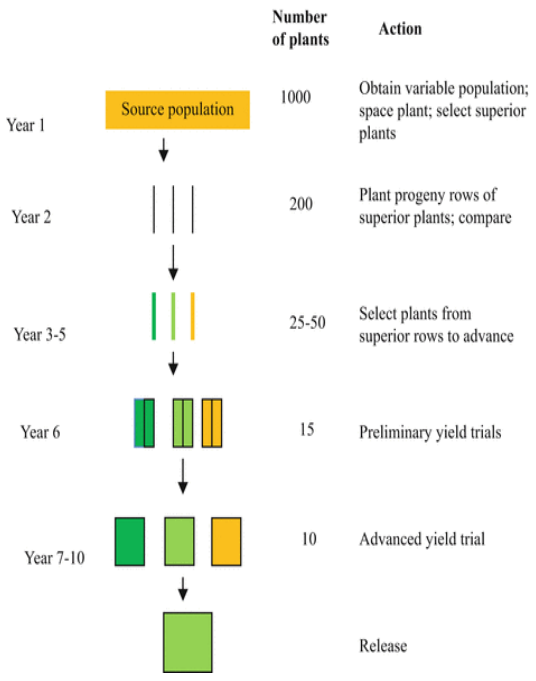

Fig. 1. Mass selection breeding method

Its agronomic requirements like optimum sowing time, seed rate and fertilizer were determined during 2017-18 to 2018-19. The candidate variety GR-722 was also evaluated for green fodder yield and mortality (%) at different salinity levels under both naturally salt-affected fields and artificially induced saline conditions during 2018-19 to 2019-20. The quality traits of green fodder were ascertained by the Biochemistry Section, AARI, Faisalabad. Spot examination of the crop was conducted during 2020. The complete development history of the variety Naimat (GR-722) is described in Table 1.

Statistical analysis

The data collected on various fodder yielding traits throughout the development period of the variety were subject to analysis of variance by using Statistix 8.1 computer statistical software package. LSD test at 5% probability level was applied to find significance among treatment means (Steel et al., 1997).

Proposed name of the variety

The name proposed for the variety is 'Naimat' subjected to the approval by the committee.

Results and Discussion

Fodder yield performance trials

Performance of the line 'GR-722' in station trials, adaptability trials and National Uniform Fodder Yield Trials is given.

Station/ regular green fodder yield trial (2014-15)

The line 'GR-722' out yielded the check variety 'Sargodha Lucerne' producing a 6.98% higher green fodder yield than the check. The line 'GR-722' gave a 7.23% higher green fodder yield than the check variety 'Sargodha Lucerne' (Table 2).

Adaptation/ zonal green fodder yield trial (2016-17)

The promising line 'GR-722' was compared with check variety 'Sargodha Lucerne' under Adaptation Yield Trials at different locations of the Punjab province during 2016-2017. The results are illustrated as in Table 3. Based on the average values of four locations, the line 'GR-722' surpassed the check variety 'Sargodha Lucerne' by producing a 7.78% higher green yield.

TABLE 1. Breeding and selection activity for variety development

Year	Activity
2007-08 to 2008-09	Random mating of lines I-Con-B, 5-IN-59, No.1103, GR-800 and No.53 to develop heterogeneous base population.
2009 – 10	Superior plants were selected from the base population, planted their clones in isolation and seed collected.
2010 -11	The bulked seed was grown and again superior plants were selected.
2011-12	Selection cycle repeated
2012-13	Selection cycle repeated
2013 -14	A uniform homogeneous population was selected for further testing in replicated trials
2014 -15 & 2015-16	Tested in replicated yield trials (Station Green Fodder Yield Trials)
2016-17	Zonal/Adaptability Green Fodder Yield Trial
2017-18	National Uniform Green Fodder Yield Trial (1st Year)/ 1st DUS Testing/ Agronomic Trials
2018-19	National Uniform Green Fodder Yield Trial (2nd Year)/ 2nd DUS Testing/ Agronomic Trials
2019-20	Spot Examination of candidate variety
2020-21	Approved as a variety namely "Naimat" by Punjab Seed Council

TABLE 2. Performance of GR-722 in Station Yield Trial during the Year 2014- 2015 at Fodder Research Institute Sargodha

Sr. No.	Name of variety	Green fodder yield (ton/ha) (2014-15)	Green fodder yield (ton/ha) (2015-16)
1	GR-722	130.2	127.5
2	Sargodha Lucerne (Check)	121.7	118.9
Increase over check		6.98%	7.23%

TABLE 3. Performance of GR-722 under adaptation/ zonal green fodder yield trial

Entry	Green fodder yield (ton/ha)				
	FRI, Sargodha	FRSS, Faisalabad	ARS, Bahawal-pur	ESPU, Farooqabad	Average
GR-722	99.31	123.5	76.65	115.09	103.63
Sargodha Lucerne (Check)	92.76	120.0	72.90	98.97	96.15
% Increase Over Check (Sargodha Lucerne)	7.06 %	2.91 %	5.14 %	16.28 %	7.78 %

National uniform fodder yield trials (2017-18 & 2018-19)

The Coordinator Fodder, NARC Islamabad tested the promising line 'GR-722' in National Uniform Fodder Yield Trials at different locations during 2017-18 & 2018-19. The results of green fodder yield at the Punjab level are shown in Tables 4, 5 and 6.

Parameters studies

Comparison of green fodder yield contributing characters of line 'GR-722' with check variety 'Sargodha Lucerne' are described in Table 7. The results of the table show that the line 'GR-722' is superior in plant height, No. of tillers/ meter and No. of leaves/ tiller to the check variety 'Sargodha Lucerne'.

Agronomic studies

Sowing date trials

The promising line 'GR-722' was sown at Fodder Research Institute, Sargodha from 1st September to 15th November at 15 days intervals during the crop years 2017-2018 and 2018-19 to find out its optimum sowing time for fodder production. The results are tabulated in Table 8 which revealed that the promising line 'GR-722' produced maximum green fodder yield (105.28ton/ha)

at a 10kg/ha seed rate. The results presented in Table 8 also indicate that the promising line 'GR-722 sown on 15th October produced maximum green fodder yield as compared to other dates of sowing.

Seed rate trials

The promising line 'GR-722' was sown at different seed rates through broadcast for maximum fodder yield during the years 2017-18 and 2018-19. The average green fodder yield of both years is given in Table 9.

Fertilizer requirements

The response of promising line 'GR-722' to different fertilizer doses was studied during the years 2017-18 and 2018-19 and the results obtained are shown in Table 10.

The green fodder yield data (Table 10) show that the promising line 'GR-722' is more responsive to fertilizer dose of 23-80-60 NPK kg/ha as it produced a maximum green fodder yield of 124.77ton/ha.

TABLE 4. Performance of alfalfa Line 'GR-722' for green fodder yield at Punjab Locations under National Uniform Fodder Yield Trial (NUFYT) during 2017-18

Entry	Average green fodder yield (ton/ha)			Average (ton/ha)	% increase from check variety
	FRSS, AARI, Faisalabad	ARS, Bahawalpur	FRI, Sargodha		
GR-722	88.00	49.33	46.93	61.42	8.15 %
Sargodha Lucerne (Check)	76.45	45.33	48.60	56.79	

TABLE 5. Performance of alfalfa Line 'GR-722' for green fodder yield at Punjab Locations under National Uniform Fodder Yield Trial (NUFYT) during 2018-19

Entry	Average green fodder yield (ton/ha)			Average (ton/ha)	% increase from check variety
	FRSS, AARI, Faisalabad	FRI, Sargodha	ARS, Bahawalpur		
GR-722	83.56	77.23	121.30	94.03	14.47 %
Sargodha Lucerne (Check)	86.67	52.17	107.60	82.14	

TABLE 6. Yield performance summary of promising Line 'GR-722' in comparison with check variety 'Sargodha Lucerne' in the Trials

Sr. No.	Type of trials	Year	No of Trials	Av. green fodder yield (t/ha)		
				GR-722	Sargodha Lucerne (check)	% increase from check variety
1.	Station Yield trials	2014-15 and 2015-2016	2	128.85	120.30	7.11 %
2	Adaptability green fodder yield trials	2016-2017	4	103.63	96.15	7.78 %
3	National Uniform Yield Trial (1st)	2017-18	3	61.42	56.79	8.15 %
4	National Uniform Yield Trial (2nd)	2018-19	3	94.03	82.14	14.47 %

TABLE 7. Comparison of green fodder yield related parameters of Line 'GR-722' with check variety 'Sargodha Lucerne'

Characters	Advance Line GR-722	Sargodha Lucerne (check)
Plant height	89cm	83cm
No. of tillers/ meter	48	39
No. of leaves/ tiller	43	41
Capsule size	Bold	Bold
1000 seed weight	2.4g	2.3g

TABLE 8. Effect of date of sowing on green fodder yield of "GR-722"

Sr. No.	Date of Sowing	Green fodder yield (ton/ha)		Average
		2017-18	2018-19	
1	1st September	80.3	86.5	83.4
2	15th September	83.2	87.1	85.15
3	1st October	96.7	99.4	98.05
4	15th October	107.1	110.4	108.75
5	1st November	101.5	105.6	103.55
6	15th November	88.3	77.2	82.75

TABLE 9. Effect of different seed rates on fodder yield (ton/ha) of "GR-722"

Sr.No.	Seed rate (kg/ha)	Green fodder yield (ton/ha)		Average
		2017-18	2018-19	
1	5	87.00	87.50	87.25
2	6.25	89.00	90.00	89.50
3	7.50	91.00	92.00	91.50
4	8.75	94.00	95.00	94.50
5	10	105.00	105.57	105.28

TABLE 10. Effect of different fertilizer levels on green fodder yield of "GR-722"

Sr. No.	Fertilizer level (kg/ha)			Green fodder yield (ton/ha)		Average
	N	P	K	2017-18	2018-19	
1.	00	00	00	104.43	110.43	107.43
2.	21	71	54	119.88	116.88	118.38
3.	22	76	57	121.55	124.55	123.05
4.	23	80	60	122.64	126.90	124.77
5.	24	84	63	120.42	120.33	120.37
6.	25	88	66	120.03	120.06	120.04

Production technology (fodder)

The optimum sowing time of promising line 'GR-722' is 15th October to obtain maximum fodder yield. The seed rate of 10kg/ha and fertilizer @ 23-80-60 NPK kg/ha is recommended to obtain economically maximum green fodder yield.

Optimum sowing date 2nd fortnight of October

Seed rate 10kg/ha

Fertilizer requirements 23-80-60 NPK kg/ha

Insect- pests and disease reaction

Insect pest reaction

The average data regarding insect pest attacks on GR-722 is mentioned as in Table 11.

The crop was kept under observation throughout the season. The insect pest was recorded negligible and predator activity was in progress.

Disease reaction

The line 'GR-722' was evaluated against the major diseases Anthracnose, Crown & Stem Rot and their behavior is given in Table 12.

The results revealed that the line 'GR-722' is moderately resistant to Anthracnose and tolerant to Crown & Stem Rot

Proximate analysis Of green fodder

The promising line 'GR-722' along with local check variety 'Sargodha Lucerne' were analyzed for dry matter, crude protein, crude fiber, and Ash (Table 13).

The results show that the promising line 'GR-722' has a higher nutritive value than the check variety 'Sargodha Lucerne' as crude protein and ash are higher than the check.

TABLE 11. Insect pest reaction (2019-20)*

Entry	Insect Pests Complex		Bio-control agents population	
	Helicoverpa larval population/ sqm	Armyworm population/ sqm	*Chrysoperia* spp. per sqm	Spotted beetle per sqm
GR-722	0.59	Nil	2.6	2.1

(*Entomological Research Institute, Faisalabad)

TABLE 12. Response of GR-722 to diseases (2019-20)*

Entry	Disease reaction	
	Anthracnose	Crown & stem rot
GR-722	Moderately resistant	Tolerant

(*Plant Pathology Research Institute, Faisalabad)

TABLE 13. Proximate analysis of fodder*

Entry	Dry matter (%)	Crude protein (%)	Crude fibre (%)	Crude fat (%)	Ash (%)
GR-722	23.30	18.10	22.60	2.03	14.15
Sargodha Lucerne (Check)	19.50	15.95	23.95	1.91	14.40

(Bio Chemistry Section, AARI, Faisalabad)*

Palatability studies

It is shown in Table 14

TABLE 14. Palatability (2017-18)

Variety	Palatability %
GR-722	83
Sargodha Lucerne (check)	80

Salinity tolerance

The promising line GR-722 along with check variety Sargodha Lucerne were evaluated against salinity under both naturally salt-affected fields and artificially induced saline conditions (Tables 15, 16). The results illustrated in Tables 15 and 16 show that the promising line 'GR-722' is comparatively salt tolerant than the check 'Sargodha Lucerne'..

Justification

1. It gives more fodder yield than the check variety Sargodha Lucerne.
2. It has more plant height, tillers/plant and leaves/tiller.
3. It has a better nutritive value than the check.
4. It has better tolerance against salinity.
5. It has better tolerance against diseases (Anthracnose & Stem/Crown rot)

TABLE 15. Performance of 'GR-722' under salted affected field condition at Pindi Bhattian*

Entry	2018-19 (EC= 8)		2019-20 (EC= 6)	
	Emergence (%)	Fodder yield (ton/ha)	Emergence (%)	Fodder yield (ton/ha)
GR-722	61	39.99	77	42.69
Sargodha Lucerne (Check)	53	26.97	69	30.78

*Soil Salinity Research Institute, Pindi Bhattian

TABLE 16. Performance of 'GR-722' under artificially induced salinity levels in pots

Salinity level	Advance Line GR-722				Sargodha Lucerne (check)			
	2018-19		2019-20		2018-19		2019-20	
	Emergence (%)	Mortality (%)	Emergence (%)	Mortality (%)	Emergence (%)	Mortality (%)	Emergence (%)	Mortality (%)
4 dSm^{-1}	54	0	94	0	40	0	91	0
8 dSm^{-1}	40	0	64	0	20	0	63	0
12 dSm^{-1}	13	0	33	0	7	0	27	0
16 dSm^{-1}	0	0	7	0	0	0	5	0
20 dSm^{-1}	0	0	0	0	0	0	0	0
24 dSm^{-1}	0	0	0	0	0	0	0	0

Conclusion

The promising line 'GR-722' was developed at Fodder Research Institute Sargodha through mass selection from base population developed by random mating of five different lines I-Con-B, 5-IN-59, No.1103, GR-800 and No.53. The uniform line 'GR-722' was selected after successive selections which were further tested under station, adaptability and national uniform fodder yield trials. In all the trials the promising line 'GR-722' out-yielded the check variety 'Sargodha Lucerne.

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