

DEVELOPMENT OF HIGH FORAGE YIELD AND BETTER-QUALITY ALFALFA POPULATION

Tariq Javaid^{1*}, Hafeez Ahmad Sadaqat¹, Muhammad Ahsan Iqbal¹ and Muhammad Ashfaq Wahid²

¹Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan, ²Department of Agronomy, University of Agriculture, Faisalabad, Pakistan

*Corresponding author's e-mail address: tariq_uaf@yahoo.com

Alfalfa is the world's best fodder due to its high protein, high calcium, low fiber and high digestibility. Experiment was planned to find out the potential in alfalfa breeding material for high biomass yield, quality traits and to understand pattern of inheritance for plant biomass and quality traits. Alfalfa genotypes were crossed with line × tester crossing design. The developed breeding material was evaluated and best cross combinations were selected. Presence of genetic variability in developed germplasm directed to exploit the better forage yield and quality related characters in alfalfa population. Combining ability analyses revealed various magnitude as well as directions.

The analysis of general combining ability showed that lines 533624, 601244, 206574, 263154 and 199279, testers 39483 and 40095 were considered as potential parents and crosses 533624 × 39483, 206574 × 39483, 533624 × 672753, 206574 × 672753, 262544 × 40095 and 37522 × 37530 were best crosses on the basis of better yield and good quality traits. Fresh plant weight, plant heights, plant dry weights and crude protein were additively controlled genetically. Total plant height, plant fresh weight, dry weights, number of nodes of each plant, its crude protein contents, ash contents and forage yield per plant may be used as vital factors for the betterment of biomass and forage yield and its quality. Selected crosses from the present study were comparable with commercial varieties. These crosses are suggested to develop the high and better yielding types in alfalfa.

Keywords: Alfalfa; forage yield, inheritance pattern, Quality parameters.

INTRODUCTION

More than 2/3rd world's population is impoverished in villages and rely on agriculture farming to fulfill its needs. Livestock breeding plays a significant role in strengthening of agricultural production in most husbandry systems. It comprises 30% area of earth (Steinfeld *et al.*, 2006). About 1.3 billion people earn their income directly from this source all over the world and 600 million growers in developing countries (Thornton *et al.*, 2006). Cattle sponsor 17% to kcal and 33% to protein intake worldwide (Rosegrant *et al.*, 2009). The main source of proteins and other nutrients for humans are milk and meat that is achieved through livestock. There is huge shortage of milk, its by-products and meat in Pakistan. For this purpose, the Govt. of Pakistan is expending more than 750 million rupees every year in the form of import bill (Government of Pakistan, 2016-17).

About 58.3 % added agricultural and 1.4 % of the GDP is shared by livestock which is a very crucial department of agriculture in Pakistan. About 8 million groups are directly engaged in raising cattle that usually have 2 to 3 buffaloes and 5 to 6 goats to drive 30 to 40 % farm income that highlights the role of livestock in the rural economy (Government of Pakistan, 2016-17). Developed countries like Australia, New Zealand, Denmark and Sweden have 1/3rd of the total number

of the cattle in the world; yet contribute about 80% towards global milk production and 66% of the global beef production. Less developed countries possess 2/3rd of the cattle but they contribute only 20% towards milk and 34% of the beef production (Govt. of Pakistan, 2016-17). The existing livestock of these countries is producing below optimum potential because of the inadequate supply of forage/fodder. The available ration for livestock is deficient in quantity as well as quality.

In Pakistan, an increase in livestock population faces fodder shortage for its rearing and production. Among the cultivated area of Pakistan, only 2.31 million hectares is under fodder cultivation producing only 51.92 million tons of forage with an average production of 22.4 t/ha. Now this area has been lessened from 2.31 million hectares to 0.198 million hectares due to preference of cereal and cash crops (Govt. of Pakistan, 2016-17). About 5648.15 tons of fodder seed was imported to fulfill this demand (Govt. of Pakistan, 2016-17).

Livestock feed is facing scarcity for the nutrients, producing a 38% gap of crude protein, 26.01 % TDN (Total digestible nutrients) (Sarwar, 2012).

Majorly two green fodder shortage periods exist in Pakistan which is May to July and November to January. To fulfill the human need for meat, dairy products for improving human health, an adequate and regular supply of palatable and better-

quality fodder is required. Therefore, the development of high yielding, better quality multi cutfodder cultivars is need of this time.

Berseem (*Trifolium alexandrinum* L.), sorghum (*Sorghum bicolor* L.), oats (*Avena sativa* L.), millet (*Panicum miliaceum* L.), jawar (*Sorghum vulgare* L.), sarsoon (*Brassica napus* L.), cowpeas (*Vigna unguiculate* L.), corn (*Zea mays* L.), sorghum-sudan grass hybrids, guar (*Cyamopsis tetragonoloba* L.), and alfalfa (*Medicago sativa* L.) are raised in Pakistan for fodder purposes. Among the traditional fodders, alfalfa probably is the world's best crop. It is an important leguminous fodder crop containing 20-24% crude protein, high calcium, phosphorus, minerals, low fiber and high digestibility. Although it is cold-loving plant yet resists high temperature. Its roots system is deep and remains in the field for a longer time that fixes nitrogen in the soil improving its fertility. Unfortunately, much work has not been done on alfalfa in Pakistan. One of the major constraints in alfalfa improvement is its narrow genetic base which is a great hurdle in breeding programs. At present almost, all the cultivar's seed of alfalfa need for planting is imported from various countries of the world at a high cost. Moreover, imported seeds are less adaptable so it is needed to develop our own promising alfalfa varieties/hybrids having high yield and good quality parameters.

Before feeding livestock feed complete quality information must be available. Feed quality is calculated in terms of performance of animal e.g. daily gain, when potential animals are given alone and free choice of feeding. Animal trials are not suitable due to expensive labour, feed and time required for screening forage genetic improvement trials. This highlights the prediction importance of small forage quality samples. Digestibility and intake of feed samples through chemical composition showed both composition of feed and nutrients availability together form nutritive value. Proteins, minerals and vitamins are the key nutrients which are required by the ruminant animals. Genetic potential of animals hinders its productivity only when there is optimum utilization of all available nutrients.

Development of high yield cultivars with good nutritional parameter, a better understanding of various characters, as well as the suitable selection of the parents, is prerequisite. The selected parents having superior traits could also produce better cultivars when crossed with other parents. The out enactment of any hybrid only relies on the specific combining ability. Combining abilities information is not only important to select good parents and crosses but also to identify way of gene action in quantitative character's expressions (Goyal and Kuamr, 1991). To meet this objective, GCA of parents must be studied to develop the improved genotype whilst SCA is examined for the betterment of crosses (Cruz and Regazzi, 1994). Various magnitude of GCA shows additive gene action and SCA shows non-additiveness or may be due to epistasis (Falconer, 1996). Therefore, gene action of the selected traits

is clearly elaborated with combining ability analysis. Different breeding methods can be utilized to find out the combining abilities and gene actions viz Line \times tester, Diallel and North Carolina etc. Due to genetic assumptions and laborious matting pattern in diallel and more number of flowers requirement in North Carolina designs (Nduwumuremyi *et al.*, 2013) make these designs not suitable. Only mating design, Line \times tester developed by Kempthorne (1957) which is effectual and can assess multiple accessions for combining ability effects and plant characters can be easily interpret on genetic basis (Bjaj *et al.*, 1997).

A multiple range of entries could be chosen to find out developed material on the basis of genetic variation which also forms the basis of plant breeding. Genetic difference as variability for an agronomic trait is the major component of efficient alfalfa breeding and to broaden its gene pool.

Objective of the present research work is to determine the potential of alfalfa germplasm for fodder and quality related parameters and also to determine the inheritance pattern of these plant characters.

MATERIALS AND METHODS

This experiment was performed in the fields of Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan during 2014-16. Accessions with good forage yield and high nutritive values were selected as lines. Poor forage yield and bad nutritive value possessed accessions were selected as testers. Fifteen lines and 4 testers were hybridized by using line \times tester breeding scheme under controlled pollinations. Hand emasulation was done followed by dusting pollens from the desired testers and covering the flowers with butter paper bags. The agronomic practices for the alfalfa crop viz fine levelled seed bed preparation, irrigation for normal crop stand to achieve full bloom and hoeings at regular intervals to eradicate weeds were carried out. Developed breeding material along with parents was stored under cool, dry, dark environment in sealed plastic bags after harvesting and cleaning for further evaluation.

Seeds of sixty crosses and their parents were grown for evaluation using triplicate alpha lattice design. Three check varieties were sown in the same field for comparison with the developed breeding material. Data of 60 crosses, their parents and also check varieties for forage yield and quality parameters viz. plant height recorded from ground level to the last node of the plant with the help of meter rod in centimeters while number of shoots recorded for each plant in each replication. Plant fresh weight was recorded with the help of electric weighing balance (Setra BL-410S) in grams while plant dry weight and leaves dry weight recorded with electric weighing balance (Setra BL-410S) after drying in oven (Isotemp. oven) at 80°C. Leaf area was calculated after recording length and width of leaves in centimeters with

meter rod while number of nodes /plants for each plant in each replication. Number of nodules /plant counted after uprooting the plant and forage yield recorded with the help of electric weighing balance (Setra BL-410S) in grams as yield traits while crude protein, acid detergent fibre, neutral detergent fibre, acid detergent lignin and ash contents were recorded after drying sample in oven (Isotemp. oven) at 80°C with NIR Analyzer (Agri NIR W Analyzer), crude fibre was recorded through distillation method as quality traits.

To check genetic variability in the breeding material, recorded data were subjected to analysis of variance (Patterson and Williams, 1976). General and specific combining abilities were determined through Line × tester breeding scheme (Kempthorne, 1957). Combining ability effects were calculated in Microsoft Excel.

RESULTS

Mean sum of squares after performing analysis of variance for different fodder yield and quality linked parameters are given in Table 1. Highly significant differences were found in all the accessions for all traits except number of shoots, forage yield and acid detergent lignin. All parents were different significantly for all parameters except for plant dry weight, number of nodes, number of nodules and acid detergent lignin. Parents vs crosses had significant differences for all traits except plant height, number of nodes, number of nodules, crude fiber, acid detergent fiber, neutral detergent fiber and acid detergent lignin. Among lines, significant differences were observed for all traits except number of shoots, plant dry weight, crude proteins, crude fiber, neutral detergent fiber and acid detergent lignin while all testers differed significantly for all the traits except plant dry weight, acid detergent fiber, acid detergent lignin and ash contents.

All the crosses showed significant difference except plant fresh weight, plant dry weight, leaf area, number of nodes and number of nodules. Line × tester interaction differed significantly for all the studied traits except leaf area, number of nodules, acid detergent fiber, neutral detergent fiber, acid detergent lignin and ash contents. Presence of genetic variability indicated that breeding material may be used further for selection and improvement of crop plants.

General Combining Ability: General combining ability for various fodder yield and quality related traits are presented in Table 2 (a) and Table 2 (b) respectively. The lines and testers had different direction and magnitude of GCA effects. Among lines, 262544 had significant GCA effects for all the traits except number of nodules and forage yield. Line 263154 had significant positive GCA effects for all the traits except plant dry weight, leaf area, number of nodes per plant, number of nodules per plant, forage yield and acid detergent fiber. Significant positive GCA effects were observed in line 199279 for all yield and quality traits except plant dry weight, leaves dry weight, leaf area, number of nodules per plant, forage yield, crude protein, acid detergent fiber and neutral detergent fiber. Lines 533624 and 601244 had positive significant GCA effects for at least six traits out of fifteen that is a great number for selection as a potential parent.

Among testers, 40095 showed significant and positive GCA effects for all traits except crude protein, acid detergent fiber, neutral detergent fiber, acid detergent lignin and ash contents. Positive and significant GCA effects were observed in tester 39483 for all traits except number of shoots per plant, leaf area, number of nodes per plant, number of nodules per plant, forage yield, crude protein, crude fiber, acid detergent fiber and neutral detergent fiber.

Table 1. Mean square values from analysis of variance for various fodder yield and quality-related traits in alfalfa accessions

SOV	Replications	Entries	Parents	Parents vs Crosses	Crosses	Lines	Testers	Line× Tester	Error
DF	2	110	18	1	59	14	3	42	188
PH	6266.35**	11800.04**	4682.63**	826.13	4408.70**	10311.00**	3611.00**	15532.00*	1194.30
NoS	3763.05**	1462.54	3648.49**	33078.00**	9591.90**	1133.60	2962.00**	14893.00*	863.32
PFW	149.04**	252.69**	109.08**	407.56**	44.00	130.62*	117.79**	467.00**	45.46
PDW	281.58	690.91*	134.14	939.28*	73.64	68.06	159.46	775.00**	228.35
LDW	25578.14**	11384.35**	50224.50**	14310.30**	12511.00**	10573.00**	15091.00**	20735.00*	3442.80
LA	13066.36**	15265.94**	21953.60**	39684.00**	2503.85	12934.00**	18127.00**	8082.47	3085.60
NoN	685.89**	406.12**	223.60	253.56	301.48	457.57**	692.71**	898.24*	140.60
NoNod	837.45**	753.13**	868.13	207.21	239.30	965.07**	558.65**	208.10	226.36
FY	13689.60**	2063.69	29316.60**	13995.41*	10503.00**	7554.92*	9332.00**	13886.00*	4409.00
CP	19762.52**	71374.05**	32302.00**	12780.01*	18651.00**	1540.76	5741.78*	10391.00*	4359.00
CF	598.10**	1743.23**	1033.38**	401.40	550.38**	4.75	34.11**	39.81**	250.37
ADF	588.20**	3377.93**	925.10**	257.47	587.06**	0.26**	0.02	0.01	246.64
NDF	26370.91**	7414.08**	7764.50**	2930.10	0.05*	66515.93	53075**	49203.40	1760.60
ADL	11283.38	4121.95	7335.93	4905.02	95566.00**	0.03	0.02	0.02	3490.40
AC	153.52**	28.90**	34.86**	24.11**	21455.00**	65784.00*	37279.89	26820.00	6.78

*=Significant at 0.05 probability level, **= Significant at 0.01 probability level, SOV= Sources of variation, DF = Degrees of freedom, PH= Plant height, NoS= Number of shoots, PFW= Plant fresh weight, PDW= Plant dry weight, LDW= Leaves dry weight, LA= Leaf area, NoN=

Number of nodes per plant, NoNod= Number of nodules per plant, FY= Forage yield, CP= Crude protein, CF= Crude fiber, ADF= Acid detergent fiber, NDF= Neutral detergent fiber, ADL= Acid detergent lignin and AC= Ash contents

Table 2. General combining ability effects of lines and testers for various fodder yield-related traits in alfalfa.

	PH	NoS	PFW	PDW	LDW	LA	NoN	No Nod	FY
Lines									
262544	-52.50*	-57.80**	-7.50**	-5.50*	-0.82**	-28.80**	-42.80**	-2.61	0.48
37502	1.72	-4.86	-1.42	2.46	-0.15	-6.68	-4.56	-0.95	3.35
37522	17.98*	-1.83	0.74	0.05	0.07	15.54*	10.56	1.30	-0.40
464766	2.24	-9.02	1.88	-0.99	0.34*	8.49	2.14	0.77	0.66
263154	18.17*	34.40**	3.79*	2.69	0.48*	3.54	10.43	0.01	-2.09
199279	12.48*	38.59**	2.53*	0.94	0.09	7.39	24.22**	1.49	-2.00
464765	-18.60*	9.54	-3.66*	0.79	-0.32	8.15	6.93	1.59	3.56*
467910	10.60*	8.91	1.81	1.94	0.29*	-14.42*	8.50	-0.98	2.08
464769	-33.60*	-25.12*	-4.28*	-4.70*	-0.05	10.74	-2.88	0.00	2.28
262544	19.54*	-3.81	0.70	-1.28	-0.12	-29.90**	-8.09	-3.10*	-2.89
549106	-12.80*	0.89	-0.19	2.79*	0.08	16.78*	5.04	1.03	-0.97
206574	34.20**	9.59	5.63**	0.61	0.15	-16.99*	-9.66	-1.61	-0.27
422246	-28.80*	-42.80**	-2.61*	0.48	0.77*	33.28**	7.09	9.60*	-0.22
533624	-6.68	-4.56	-0.95	3.35*	0.01	8.15	6.93	1.59	3.56*
601244	15.54*	10.56	1.30	-0.40	1.49*	24.16*	66.30**	3.10*	2.55
SE	9.45	11.14	2.06	2.70	0.27	13.83	13.09	2.79	3.55
Testers									
672753	-8.02	-22.98*	-0.71*	0.06*	-94.43*	-10.27	-0.25*	-0.6*	-11.00*
37530	-1.80	0.46	-0.41	0.02	-36.90*	-17.94*	-0.03	-0.30	-24.00*
39483	10.48*	-14.34*	2.14**	0.05*	50.57*	7.53	-0.24	0.04	3.88
40095	35.70**	30.65*	0.80*	-0.90*	109.30**	26.28*	-0.16	-0.28	18.60*
SE	9.89	13.93	0.61	0.03	38.60	11.49	0.19	0.51	9.89

SE= Standard Error, *= Significant at probability level of 0.05, **= Significant at probability level of 0.01, PH= Plant height, NoS= Number of shoots, PFW= Plant fresh weight, PDW= Plant dry weight, LDW= Leaves dry weight, LA= Leaf area, NoN= Number of nodes per plant, NoNod= Number of nodules per plant, FY= Forage yield

Table 2. (b) General combining ability effects of lines and testers for various fodder quality-related traits in alfalfa.

	CP	CF	ADF	NDF	ADL	AC
Lines						
262544	-52.67**	-50.47**	-6.09*	-3.54*	-14.39*	7.21*
37502	13.55*	-22.64*	-0.49	-3.70*	11.07*	26.49*
37522	12.82*	-26.14*	-0.40	1.67	-40.41*	-49.94**
464766	-10.48*	-17.76*	-0.88	-1.45	14.63*	-6.51*
263154	12.62*	50.70**	4.74	4.46*	-10.81*	12.02*
199279	-27.09*	12.90*	-4.80	1.83	39.92*	10.72*
464765	9.90*	3.13	1.48	-0.91	15.65*	15.56*
467910	-31.05*	-17.32*	-4.98	-6.23*	-5.21*	-1.54
464769	27.20*	-9.96	1.53	-0.06	3.95*	4.47*
262544	-8.43	0.30	1.32	4.82*	-4.76*	-5.15*
549106	29.47*	10.95	5.44	0.55	-0.47	-2.81
206574	13.83*	13.09*	2.79	3.55*	-0.28	3.54
422246	-76.84**	-78.58**	-13.85**	-12.40**	6.76*	1.49
533624	-1.71	12.62*	-2.82	7.73*	3.79*	3.79*
601244	25.60*	10.08	1.33	-1.13	2.99	2.28
SE	8.71	-11.44	5.74	2.17	3.73	3.70
Testers						
672753	16.24*	0.13	-0.04*	11.77	17.06*	0.48*
37530	-10.03	-1.95**	-0.05*	-40.32	-22.66*	0.29*
39483	-3.41	-0.07	0.02	-26.20	23.92*	0.48*
40095	9.86	1.58*	0.02	30.77	3.81	-0.55**
SE	13.93	0.61	0.03	38.60	11.49	0.19

Development of better alfalfa population

SE= Standard Error, *= Significant at probability level of 0.05, **= Significant at probability level of 0.01, CP= Crude protein, CF= Crude fiber, ADF= Acid detergent fiber, NDF= Neutral detergent fiber, ADL= Acid detergent lignin and AC= Ash contents

Table 3. (a) Specific combining ability effects of crosses for fodder yield-related traits in alfalfa.

Crosses	PH	NoS	PFW	PDW	LDW	LA	NON	NoNod	FY
262544x672753	32.74	3.51	11.51	0.35	-0.83	-0.10	1.59	-2.73	97.32
262544x37530	-8.42	-54.25*	-27.94	-8.35	-4.57	-0.37	-11.61	-12.43	-19.88
262544x40095	3.72	16.18	9.37	8.11	1.09	0.75	3.03	-8.41	105.48
262544x39483	-63.68*	-16.94	-37.82	-4.80	-4.57	-0.59	-0.82	3.98	-109.41
37502x672753	55.52*	-15.38	-8.84	-4.33	2.18	-0.44	2.36	18.40*	60.29
37502x37530	-19.87	66.88**	53.72	9.02	6.70	0.75	5.46	1.18	-133.80
37502x40095	3.60	-4.94	12.57	-6.68	3.80	-0.53	-9.09	-2.99	-6.82
37502x39483	-26.64	-76.9**	-67.78*	-11.35*	-13.30*	-0.38	-17.89*	-18.22*	13.48
37522x672753	-2.25	6.04	7.63	-0.78	1.06	-0.30	-5.51	8.20	-89.66
37522x37530	-27.09	10.56	0.91	17.64**	6.10	1.64*	22.23**	8.93	-6.22
37522x40095	-4.69	42.08	5.59	7.11	-3.87	0.12	10.14	-12.72	-29.68
37522x39483	57.06*	22.34	41.08	-5.92	6.21	-0.55	0.11	16.79	118.90
464766x672753	52.63*	4.72	7.79	-0.08	-0.95	0.22	0.37	-2.67	109.50
464766x37530	-8.89	32.25	8.14	11.01*	5.15	1.37*	9.37	6.56	137.97
464766x40095	-8.97	-12.73	-25.00	-7.69	-5.28	-1.50*	-1.46	-6.69	48.00
464766x39483	67.06**	-22.56	-48.81	-5.67	-5.94	-0.30	-6.24	-14.03	-121.16
263154x672753	-40.79	-2.47	51.17	2.09	11.12	0.69	-3.87	18.73*	-127.36
263154x37530	-61.05*	0.79	6.71	0.35	-4.11	-0.42	1.83	-1.90	-46.95
263154x40095	-12.62	6.96	0.14	6.25	7.66	0.21	7.32	19.09*	-88.79
263154x39483	-70.20**	-12.95	-33.08	-1.00	-8.33	-0.54	3.12	-7.21	-121.65
199279x672753	-8.69	-0.68	30.29	2.15	12.75	-0.36	11.90	24.48**	-56.96
199279x37530	18.89	23.06	38.50	-0.14	-1.01	0.40	-10.02	-13.93	201.80*
199279x40095	3.55	-33.69	-50.98	-11.36*	-12.87	0.15	-15.91*	-18.57*	-18.65
199279x39483	69.14**	17.29	15.13	4.10	1.79	0.15	3.59	-3.86	84.27
464765x672753	-75.4**	-8.42	-30.24	-0.77	-9.13	0.12	-3.33	-9.52	-53.43
464765x37530	53.57*	95.71**	117.30**	14.98**	26.66**	0.12	19.47**	32.11**	-95.63
464765x40095	7.01	-27.09	-15.92	-7.45	-12.71	1.34*	-13.36	-22.06*	-49.93
464765x39483	-30.02	26.59	59.72*	-0.21	11.95	-0.56	3.79	31.19**	51.17
467910x672753	82.77**	-59.08*	-75.40**	-10.76*	-15.44*	-1.09	-15.03*	-28.7**	214.70*
467910x37530	-37.96	-27.70	-55.34*	4.21	-1.32	0.07	8.47	-3.01	-66.88
467910x40095	-0.98	-1.84	-1.77	0.92	-0.55	0.08	3.14	-1.19	-57.77
467910x39483	60.65*	15.34	3.54	-5.29	-5.61	-0.18	-2.46	-0.82	85.70
464769x672753	9.17	18.28	-6.37	5.66	3.09	0.13	5.39	4.47	43.07
464769x37530	34.83	-20.71	-12.50	-6.81	-6.52	-0.58	-8.93	-16.14	-16.16
464769x40095	-96.40**	68.54**	78.40**	17.26**	18.87**	1.57*	22.31**	22.88*	-99.30
464769x39483	-7.31	-79.60**	-61.31*	-11.75*	-9.27	-0.01	-19.6**	-9.21	44.45
262544x672753	-89.90	78.43**	23.50	2.30	2.60	0.021	1.19	2.75	24.50
262544x37530	98.76	65.67**	56.50*	4.30	3.50	0.03	0.09	2.23	45.70
262544x40095	56.67	67.40**	48.60*	6.78*	3.60	0.01	1.99	1.70	56.40
262544x39483	77.80	56.87**	55.40*	5.50*	6.12	0.07	2.98	3.86	78.50
549106x672753	73.50	24.60**	39.50*	4.20	5.60	0.04	3.87	4.47	67.50
549106x37530	56.40	12.70	44.50*	3.30	1.99	0.09	4.80	7.89	78.60
549106x40095	57.30	-10.60	39.40*	2.87	2.98	0.076	9.98*	16.50*	67.50
549106x39483	34.90	11.80	43.50*	2.90	3.98	0.05	4.87	15.60*	45.30
206574x672753	89.50	8.60	22.80	3.60	4.98	0.98*	5.98	14.87*	99.98*
206574x37530	46.80	78.60**	34.50*	4.80	6.42	0.09	8.87*	12.87*	99.40
206574x40095	46.70	45.60**	25.30	3.90	5.89	0.063	9.98*	3.40	101.20**
206574x39483	32.40	67.80**	34.50*	2.30	6.98*	0.02	8.89*	5.89	56.90
422246x672753	26.40	45.70*	45.70*	4.40	9.87*	0.61	9.87*	4.98	56.40
422246x37530	78.60	46.80*	4.70	12.30*	13.20*	0.09	13.50*	6.70	34.60
422246x40095	57.50	14.50	9.80	22.30*	9.90*	0.08	12.50*	5.89	34.60
422246x39483	46.40	27.60*	13.20	5.60*	7.90*	0.01	9.98*	4.50	112.40**
533624x672753	78.40	44.30*	34.89*	4.90	12.80*	0.008	7.98*	5.87	98.80*
533624x37530	89.60	49.70*	45.70*	3.40	5.60	0.01	5.98	4.98	78.50
533624x40095	99.40	54.50*	67.50**	2.30	4.60	0.003	4.89	5.98	67.50
533624x39483	46.30	68.60*	14.91	6.90*	7.90*	0.98*	5.98	6.70	56.50
SE	24.22	23.15	27.29	5.04	6.62	0.67	6.85	8.69	94.55

SE= Standard Error, PH= Plant height, NoS= Number of shoots, PFW= Plant fresh weight, PDW= Plant dry weight, LDW= Leaves dry weight, LA= Leaf area, NoN= Number of nodes per plant, NoNod= Number of nodules per plant, FY= Forage yield.

Table 3. (b) Specific combining ability effects of crosses for fodder quality-related traits in alfalfa.

Crosses	CP	CF	ADF	NDF	ADL	AC
262544x672753	0.58	2.32	4.27	-2.91	-2.84	-5.83
262544x37530	0.16	-4.34	-7.89	-7.21	6.08	1.04
262544x40095	-0.32	-2.42	2.49	-2.28	-4.18	0.79
262544x39483	0.87	-3.21	-2.87	3.46	-3.59	2.84
37502x672753	-0.87	7.74	5.15	2.62	7.52	3.20
37502x37530	-0.43	-0.10	-1.15	6.33	-2.99	-2.03
37502x40095	0.69	0.00	-5.58	-4.02	6.13	0.63
37502x39483	0.49	-1.45	8.87	11.91*	-1.11	2.59
37522x672753	0.98	-3.68	-1.08	0.83	7.16	-1.33
37522x37530	-1.43	3.09	5.35	2.54	-1.35	-3.94
37522x40095	1.20	-6.41	-1.99	-6.57	-5.31	-2.67
37522x39483	-1.93	8.44	-5.56	-4.69	-5.52	4.73
464766x672753	0.73	-2.20	-0.72	2.84	-0.53	3.30
464766x37530	-0.42	7.29	4.19	3.31	-7.34	-4.29
464766x40095	1.14	9.30	-2.32	-3.53	-1.53	-4.96
464766x39483	0.36	-7.50	0.78	1.81	0.78	6.66
263154x672753	-0.65	-1.56	-0.30	-2.97	4.40	1.30
263154x37530	-1.15	-5.32	-1.63	-1.46	4.22	-2.01
263154x40095	-0.33	-0.10	-0.63	-2.52	-2.60	3.42
263154x39483	0.16	6.08	-2.39	2.77	0.12	0.34
199279x672753	-0.18	1.46	2.69	5.40	0.26	3.52
199279x37530	-0.52	9.35	1.26	-3.99	5.84	-7.30*
199279x40095	0.15	-8.28	-2.28	-7.77	2.35	-0.50
199279x39483	0.74	-8.50	1.35	6.11	-5.96	0.55
464765x672753	-0.92	-5.05	4.27	8.94	-6.33	-2.36
464765x37530	-0.58	-3.20	1.14	-3.60	5.60	1.82
464765x40095	0.21	-2.11	1.13	-1.27	-0.83	2.42
464765x39483	-0.38	-3.10	-3.34	-7.27	3.12	1.58
467910x672753	0.18	1.38	-6.25	1.66	-6.87	0.82
467910x37530	1.49	12.90*	3.05	1.54	5.32	-4.28
467910x40095	-0.74	5.04	-1.61	-2.33	6.16	0.85
467910x39483	0.19	-4.39	-3.93	-7.17	-3.35	-1.51
464769x672753	-1.83	-2.54	-2.91	0.86	-0.88	-0.43
464769x37530	1.11	1.37	-1.18	3.46	-4.79	0.19
464769x40095	-0.01	7.12	5.68	13.02*	-2.08	-2.14
464769x39483	1.28	-6.61	3.94	-7.83	4.94	3.05
262544x672753	0.29	7.80	6.90*	3.87	2.01	3.40
262544x37530	1.23	8.90*	7.90*	2.98	1.98	1.20
262544x40095	0.25	5.90*	8.90*	4.50	2.29	2.30
262544x39483	0.45	9.80*	7.80*	2.90	3.20	6.74*
549106x672753	0.98	2.30	9.90*	3.20	3.98	5.48*
549106x37530	0.76	0.45	7.90*	1.40	4.98	7.4*
549106x40095	0.25	0.99	9.80*	7.90*	5.60	5.75*
549106x39483	1.01	1.35	3.20	5.98*	10.20*	5.60*
206574x672753	1.09	1.98	3.70	6.70*	9.87*	9.87*
206574x37530	2.34*	2.20	2.98	0.87	6.70	7.89*
206574x40095	1.98*	2.76	7.90*	2.98	7.60*	6.79*
206574x39483	0.01	2.90	9.90*	2.70	6.70*	5.67*
422246x672753	0.87	3.70	8.90*	3.60	5.70	4.50*
422246x37530	0.09	3.98	2.90	9.90*	9.98*	2.50
422246x40095	3.45*	4.50	3.60	7.98*	8.89*	6.70*
422246x39483	6.70*	6.50*	4.70	6.98*	3.98	9.87*
533624x672753	7.87*	7.98*	5.60	5.90*	3.32	8.89*
533624x37530	5.60*	8.60*	6.40*	9.90*	2.98	7.89*
533624x40095	3.98*	8.90*	6.70*	7.80*	3.98	6.50*
533624x39483	4.50*	6.90*	7.90*	6.98*	9.87*	5.60*
SE	1.25	5.70	6.11	5.63	7.08	3.53

SE= Standard Error, CP= Crude protein, CF=Crude fiber, ADF=Acid detergent fiber, NDF= Neutral detergent fiber, ADL=Acid detergent lignin, AC= Ash content

Specific Combining Ability: Specific combining ability effects of crosses for various fodder yield and quality related traits are presented in Table 3 (a) and Table 3 (b), respectively. The results showed variable magnitude and direction of SCA for various traits among crosses. Cross 533624 × 37530 had significant SCA effects for all the traits except plant height, plant fresh weight, number of nodes per plant, number of nodules per plant and forage yield. Significant SCA effects were observed in crosses 533624 × 672753 and 422246 × 37530 for all the yield and quality traits except plant height, plant fresh weight, plant dry weight, leaf area, number of nodules per plant, acid detergent fiber and acid detergent lignin. Cross 464769 × 40095 had significant SCA effects for all the yield and quality traits except forage yield, crude protein, crude fiber, acid detergent fiber, acid detergent lignin and ash contents. Significant SCA effects had been shown by the cross 464765 × 37530 for all the traits except leaf area, forage yield, crude protein, crude fiber, acid detergent fiber, neutral detergent fiber, acid detergent lignin and ash contents. Significant SCA effects were exhibited by the cross 206574 × 40095 for all the yield and quality traits except plant height, plant fresh weight, plant dry weight, leaves dry weight, leaf area, number of nodes per plant, crude fiber and neutral detergent fiber. Cross 206574 × 37530 showed significant SCA effects for most of the yield and quality traits except plant height, plant dry weight, leaf area, number of nodules per plant, forage yield, crude protein, crude fiber and neutral detergent fiber. Significant SCA effects had been exhibited by the cross 422246 × 40095 for all the yield and quality traits except plant height, number of shoots, plant fresh weight, leaf area, number of nodules per plant, forage yield, crude fiber and acid detergent fiber. Crosses 549106 × 40095, 206574 × 672753, 206574 × 39483, 422246 × 672753 and 422246 × 39483 had positive and significant SCA effects for yield parameters like number of shoots, plant fresh weight, leaf dry weight, number of nodules and also for quality parameters like neutral detergent fiber, acid detergent lignin and ash contents.

These crosses with high positive SCA effects showed the presence of heterosis and may be used in the development of hybrid seed production. Four types of gene action were also observed for selected crosses i.e. Low GCA of line × High GCA of the tester, High GCA of line × Low GCA of the tester, Low GCA of line × Low GCA of the tester and High GCA of line × High GCA of the tester.

DISCUSSION

Livestock is a very important sector in agriculture. It is increasing day by day but its production potential is very low in Pakistan just like other developing countries. Resultantly dairy products like milk and meat are also being imported by

spending huge amount (Bilal *et al.*, 2006). In developed countries, livestock population is low as compared to developing countries like Pakistan but they have great potential to feed their own countries and also sharing livestock products in global export. The fodder crop group is neglected in Pakistan and growing area under fodder crops is reducing up to 2% in each decade (Sarwar *et al.*, 2002). Alfalfa is one of the most important forages that secures fodder availability to the livestock during the lean period ensuring sustainable production from the livestock (Ammanullah, 2007). According to the current situation, biomass could be the cheapest source for agriculturist countries and its production has many merits in daily routine life. An ideotype crop for good forage yield and good quality has high yield, low moisture, ash contents, high level of fiber contents and good performance under different conditions (Tahir *et al.*, 2011). Alfalfa is such crop with plenty of desirable traits for high biomass production (Jacobsen *et al.*, 1992). It can help to maintain forage production level in that period when production of quality forage decreases due to unfavorable conditions (Tahir *et al.*, 2005). But unfortunately, in Pakistan, it is the most ignored crop due to the preference of other crops. If alfalfa is used for the fodder purpose in Pakistan, it will help the country to solve the issues of fodder shortage.

Genetic variability is a vital component for an efficient plant breeding programme (Arunkumar *et al.*, 2014). Sufficient genetic variation is necessary to improve or screen alfalfa accessions for high forage yield and best quality traits (Sprague, 1966). Genetic variation among the developed breeding material and their parents were observed for most of the forage yield and quality characters. It indicated that this breeding material can be used in the breeding program for the improvement of biomass yield and forage quality traits in alfalfa. Selection would be effective if characters correlate with each other (Smart *et al.*, 2003). Presence of genetic variability for all the studied traits proposed that heterosis is present in crosses for these traits so, selection could be more effective. Kadam *et al.* (2000) and Mohammed (2007) found highly significant differences between parents and crosses for forage yield, which showed the presence of heterosis in the crosses for forage yield. The selection of parental lines was one of the objectives of this study. The estimates of GCA effects aid in the selection of superior genotypes as parents for breeding programmes. Lines 533624, 601244, 206574, 263154 and 199279, testers 39483 and 40095 had positive significant GCA effects for maximum traits. The selected line and testers also have high mean values for most of the traits and high GCA effects indicated that character is less influenced by its mean and more related to intrinsic genetic makeup of the genotypes (Kenga *et al.*, 2004; Cruz and Reggazzi, 1994) and selection could be effective in early

generation (Roy *et al.*, 2002). Low GCA effects indicated that mean of a parent in crosses does not differ greatly from the general mean of crosses (Markinkovic, 1993). The presence of high GCA with additive effects suggest a wide adaptation and high potential of the lines for use as parents in developing widely adapted hybrids with high biomass yield and nutritional quality (Kenga *et al.*, 2004). Selected lines and testers were the best general combiners for most of the traits and may be used in the development of alfalfa types for higher forage yield and good quality attributes.

Specific combining ability effects represent both dominant and epistatic gene actions. In this study, crosses 533624 × 39483, 533624 × 672753, 422246 × 39483, 464769 × 40095, 464765 × 37530, 206574 × 40095, 206574 × 39483, 422246 × 40095, 533624 × 37530, 533624 × 40095, 262544 × 39483, 549106 × 40095, 206574 × 672753, 206574 × 37530, 42246 × 37530, 262544 × 40095 and 549106 × 39483 had maximum positive significant SCA effects for most of the traits including forage yield and quality related parameters. SCA effects alone have limited value for parental choice in a breeding program (Marilia *et al.*, 2001), therefore it is suggested that the SCA effects should be used in combination with other parameters, such as GCA of the respective parents. Four types of crosses were observed i.e. high GCA effect of line and high GCA effect of tester (533624 × 39483 and 206574 × 39483), low GCA effect of line and high GCA effect of tester (262544 × 40095), high GCA effect of line and low GCA effect of tester (533624 × 672753 and 206574 × 672753) and low GCA effect of line and low GCA effect of tester (37522 × 37530). The concentration of favorable alleles would increase with promising SCA effects in which at least one of the parents shows high GCA which is a significant situation (Kenga *et al.*, 2014). Three crosses from present studies 262544 × 40095, 533624 × 672753 and 206574 × 672753 showed significant results for better yield and good quality traits and these may be used further for the cultivar development. Selection of these crosses with useful traits is an excellent choice. Preponderance of additive genes are shown by the crosses with high SCA effects for different characters in which one or both parents with good general combining ability are involved. Like crosses 533624 × 39483 and 206574 × 39483 had additive genetic effects and selection is the best choice here. While non-additive gene action is present when both parents of the cross had lowest GCA effect such as 37522 × 37530. It showed non-additive gene action and lead towards hybrid seed production.

High SCA effects shown by the hybrids, have been resulted by crossing high × high and high × low general combiners. Interaction of good and poor combiners which contributes dominant and recessive alleles may result in hybrids with high SCA.

Parents with low or non-significant GCA effects resulted in positive and significant SCA effects showed the significance

of non-additive genetic effects controlling the expression of these characters. Parents with significant SCA effects with low general combining ability resulted in crosses with significant positive SCA effects may be improved with single parent selection in later generations.

Conclusion: Genetic variation among entries indicated that this breeding material may be further used for the improvement of biomass yield and forage quality-related traits. Differences among parent's vs crosses for various traits proposed that heterosis is present in crosses for these traits. Lines 533624, 601244, 206574, 263154 and 199279, testers 39483 and 40095 had positive significant GCA effects for maximum traits and considered as best general combiners. The crosses had variable magnitudes and directions of specific combining ability effects for the traits studied. These crosses also significantly exceeded the check varieties for most of these traits. These crosses may be used as a potential source for the improvement of alfalfa for biomass and good quality traits.

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