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Full Length Article

Performance Evaluation of a New Multifoliate Inbred Alfalfa (*Medicago sativa*) Variety via Space Mutation Breeding

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Abstract

The advantages and disadvantages of alfalfa varieties affected the establishment and production performance of alfalfa. In order to screen out the excellent varieties for experimental research and production, a new alfalfa (*Medicago sativa* L.) variety Zhongtian No. 1 (ZT1) was developed by space mutation with microgravity, high vacuum and strong cosmic radiation. Four different alfalfa varieties were used as control. Design of experiment was randomized complete block and the comparison was made for the productivity and quality of ZT1. The experiment was conducted at two field locations on the Loess Plateau in north-central China. The basic characteristics of ZT1 were high quality and high yield, in accordance with high multifoliaolate rate and high nutrition. The leaflet number of ZT1 was 5 mainly, and the multifoliaolate rate was 41.5%. Furthermore, the hay yield of ZT1 was 15,508.4 kg·ha^{-1}, which was 12.69% higher than control varieties. The CP content was 20.08%, which was 2.97% higher than control varieties, and AAs content was 12.32%, which was 1.57% higher than controls. The results revealed that ZT1 was valuable for improving the yield and quality of alfalfa, which owned the resources advantages in alfalfa industrialization, and had wide application prospect.

Keywords: *Medicago sativa*; Multifoliate trait; Space mutation; Yield and quality trials

Introduction

As the forage legume with the highest acreage in the world, alfalfa (*Medicago sativa* L.) is known as the “queen of the forages” (Lei *et al.,* 2018). Alfalfa enhances soil fertility and fixes 135-605 kg/hm^2 atmospheric nitrogen per year. In addition, alfalfa is a suitable source for biofuel production with the perennial nature and rapid biomass accumulation to meet the energy requirements of the increasing world population (Arshad *et al.,* 2017).

However, alfalfa is challenging to breed and improve, because of its complex genetic background and flowers are cross-pollinated (Chang *et al.,* 2012). In addition, conventional breeding methods are often limited by long breeding cycles, while transgenic breeding is limited by its technically specialized and costly processes (Wang *et al.,* 2009; Sun *et al.,* 2013). As an alternative, space mutation breeding is a novel and rapidly developing technique that combines advanced space technology with conventional breeding (Liu, 2007; Ren *et al.,* 2010; Yang *et al.,* 2013). Mutations are induced by multiple factors, such as microgravity, high vacuum, and strong cosmic radiation. Space mutation breeding has become an important part of breeding programs in China (Ren *et al.,* 2008; Xu *et al.,* 2009; Shi *et al.,* 2010). Compared to conventional breeding, space mutation breeding has the advantage of high mutation rates, extensive mutation range, and short breeding cycles (Du, 2010; Fan, 2010; Yang *et al.,* 2015).

Zhongtian No. 1 (ZT1), is the first alfalfa variety developed using space mutation breeding in China (Yang *et al.,* 2019). The variety was certified by the Grass Variety Certification Committee of Gansu Province in January 2014, registered as an inbred variety “Hangmu No. 1” (Accession number: GCS014). In 2018, *M. sativa* cv. Zhongtian No.1 has been listed as a species and approved by the Chinese Herbage Cultivar Registration Board (Accession number: 535). The objective of the present study was to evaluate the agronomic performance and nutrient content of ZT1 against that of four elite varieties in semi-dry and semi-humid areas of the Loess Plateau in China, over a period of three years.

Materials and Methods

In the present study, the agronomic performance of alfalfa variety ZT1 (a new alfalfa variety), which was developed *via* space mutation breeding and conventional selection methods, was compared to that of four other elite varieties

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that were used as controls (Fig. 1). In March 2002, *M. sativa* cv. Sanditi seeds were sent into space on the Shenzhou 3 spacecraft. The flight lasted for 7 days (25^{th} March–1^{th} April) in a near-Earth orbit (198–338 km), with a tilt angle of 42.4°. The spacecraft orbited the Earth for 108 cycles. From 2003 to 2010, ZT1 with multifoliaolate leaflets was selected through 4 cycles and exhibited the stable characteristics. The controls were "Sanditi", which was developed by Barenbrug, "Zhonglan No. 1", an elite variety in China, "Longdong", another elite variety in China, and "Concett", a Canadian multifoliate variety.

ZT1 alfalfa was evaluated against the control varieties for three years (2011–2013) at two locations, Lanzhou and Gangu, on the Loess Plateau, China. Lanzhou (103°44′36″ E, 36°02′20″ N, 1697 m above sea level) is a typical semi-arid area on the south shore of the Yellow River and has an average annual precipitation of 324.5 mm, an annual evaporation rate of 1450 mm, and relative humidity of 58%. The average annual temperature is 9.3°C (maximum temperature, 39.1°C; minimum temperature, −23.1°C), and the active accumulated temperature ≥ 0°C is 3,700°C and that ≥ 10°C is 1,900–2,300°C. The frost-free period lasts for 169 d, and there are 2751.4 h of total annual sunshine. The soil is classified as Grade III self-weight collapsible loess (Chang *et al.,* 2012; Yang *et al.,* 2013; 2015). Gangu (104°59'–105°30' E, 34°31'–35°03' N, 1280 m above sea level) is a typical semi-humid area in the midsouthern Long region and has an average annual precipitation of 473.1 mm and an annual evaporation rate of 1519.9 mm. The average annual temperature is 10.2°C, with ~2131 h of total annual sunshine, and the frost-free period lasts for ~187 d. The soil is derived from loess (Chang *et al.,* 2012; Yang *et al.,* 2013).

Sixteen blocks were designed of four varieties in each region; every variety was arranged in a randomized block design. In Lanzhou, the plots size was 2.7 m × 7 m, with 30 cm row to row distance, seeding rate was 2.2 $g{\cdot}m^{-2}$ (41.6 g per block). Each plot was separated from the next by 1 m wide strip. The varieties that were evaluated including ZT1, "Sanditi", "Zhonglan No. 1," and "Concett". In Gangu, the plots were 3×5 m, with 30 cm row to row distance, seeding rate was 2.2 $g{\cdot}m^{-2}$ (33.0 g per block). Each plot was separated from the next by 1.5 m wide strip. The varieties that were evaluated including ZT1, "Sanditi", "Zhonglan No. 1", and "Longdong".

We established three new indicators for evaluating multifoliate rate (MFR), which included branch multifoliate rate (BMFR), leaf multifoliate rate (LMFR), and variety multifoliate rate (VMFR). BMFR was calculated as the percentage of branches with multiple leaves (i.e., multifoliate) and was measured by dividing each experimental plot into 1 m^2 quadrats by randomly selecting three quadrats. All plants were cut at 6 cm above the ground, and counted the number of multifoliate and regular branches. Meanwhile, LMFR was calculated as the percentage of leaves on a branch with more than three leaflets (i.e., multifoliate) and was measured using 20 multifoliate branches that were randomly selected from the multifoliate branches identified during BMFR evaluation. VMFR was then calculated as the product of BMFR and LMFR.

Fig. 1: Characteristics of "Zhongtian No. 1" alfalfa leaves
A, Normal leaf with five leaflets. **B**, Palmately compound leaf with five leaflets. **C**, Abnormal leaf with five leaflets. **D**, Normal leaf with six leaflets. **E**, Pinnate leaf with six leaflets. **F**, Pinnate leaf with seven leaflets

Growth period, plant height, hay yield (HY), the ratio of dry weight to fresh weight (DW/FW), and the ratio of leaf number to branch number (L/B) were also evaluated. All measurements followed the Agriculture Trade Standards of China, namely "The code of practice for regional trials of forage grasses". The contents of nutrients, amino acids (AAs), were measured by the China National Feed Quality Control Center (Beijing). The water contents were determined from ratio of fresh to dry weight. The crude protein content (CP) was measured by Kjeldahl method, the crude fiber content (CF) was measured by acid alkali washing method. The ether extract (EE) was taken by Soxhlet extraction. Ash content was determined by method of burning (removal of carbon) at high temperature. Calcium (Ca) was determined by potassium permanganate titration, phosphorus (P) was determined by the molybdenum yellow colorimetric method, and nitrogen-free extract (NFE) was measured by indirect calculation method. In addition, trace elements including Zn, Mn, Cu, Fe and Mg were measured using flame atomic absorption spectrometry, and B was estimated using ultraviolet spectrophotometry at the Institute of Quality Standard and Testing Technology for Agro-Products of the Chinese Academy of Agricultural Sciences (Gansu).

All the data were subjected to one-way analysis of variance (ANOVA), using SPSS 13.0. Duncan's Multiple Range Test (DMRT) was used to detect significant differences between means at a significance level of $P <$

0.05 and to evaluate obvious significant differences at a significance level of $P < 0.01$. The linear correlations of 11 parameters (MFR, HY, seed yield, SY, CP, CF, AAs, Zn, Mn, Cu, Fe, and Mg) were also analyzed using SPSS 13.0.

Results

All the varieties exhibited similar growth at both experimental sites. In Lanzhou, seeds were planted in mid-April, the periods of germination, tillering, jointing, blossoming and maturation were late-April, mid-June, early-July, mid-July, and mid-August to mid-September, respectively. In Gangu, seeds were planted in early April, the periods of germination, tillering, jointing, blossoming and maturation were mid-April, mid-June, late-June, mid-July, and mid-July to mid-August, respectively.

The ZT1 plants exhibited BMFR, LMFR and VMFR values of 83.49, 41.5 and 34.64%, respectively (Table 1), whereas those of "Concett" plants were 30.58, 10.7, and 3.27%. Both "Sanditi" and "Zhonglan No. 1" plants failed to produce any multifoliate branches (MFR = 0). In addition, the MFR values of ZT1 were significantly higher than those of "Concett" ($P < 0.01$). Furthermore, the mean L/B of ZT1 was significantly higher than that of the control varieties ($P < 0.05$; Table 1), and the mean DW/FW of ZT1 (50.36%) was 5.27% higher than that of the control varieties (47.97% in "Sanditi," 47.67% in "Zhonglan No. 1," and 47.27% in "Concett").

In Lanzhou, the average plant height of ZT1 was 89.7 cm, which was 10.84% higher than that of the control varieties, and the average hay yield of ZT1 was 14,237.5 $kg \cdot ha^{-1}$, being 14.75% higher than that of "Sanditi," 6.37% higher than that of "Zhonglan No. 1," and 19.45% higher than that of "Concett." Overall, the hay yield of ZT1 was 13.26% higher than controls (Table 2). In Gangu, the average plant height of ZT1 was 99.2 cm, 10.63% higher than that of the control varieties, and the average hay yield of ZT1 was 16,779.3 $kg \cdot ha^{-1}$, 16.07% higher than that of "Sanditi", 6.46% higher than that of "Zhonglan No. 1," and 14.60% higher than that of "Longdong." Overall, the hay yield of ZT1 was 12.21% higher than controls (Table 3).

In Lanzhou, the seed yield of ZT1 was 289.6 $kg \cdot ha^{-1}$, being 1.90% higher than that of "Sanditi" (288.8 $kg \cdot ha^{-1}$), "Zhonglan No. 1" (298.1 $kg \cdot ha^{-1}$), or "Concett" (265.6 $kg \cdot ha^{-1}$); and in Gangu, the seed yield of ZT1 was 262.2 $kg \cdot ha^{-1}$, which was 1.80% higher than that of "Sanditi" (268.6 $kg \cdot ha^{-1}$), "Zhonglan No. 1" (286.2 $kg \cdot ha^{-1}$), or "Longdong" (246.2 $kg \cdot ha^{-1}$). However, neither of the difference was significant (Table 4).

At the first harvest, the CP of ZT1 was 20.08% which was 2.97% higher than that of the control varieties. At the second harvest, the CP of ZT1 (18.42%) was also higher than that of the control varieties, by 5.79%. In ZT1, the CF (7.19%), ether extract (EE; 14.76%), ash content (1.17%), Ca (9.96%), P (26.62%), nitrogen-free extract (NFE; 8.84%), and the water (4.05%) contents were all higher than those of the control varieties (Table 5).

The total content of the 18 essential AAs was 12.32% in ZT1, 11.67% in "Sanditi," 12.52% in "Zhonglan No. 1," and 12.19% in "Concett". Overall, the total AA content in ZT1 was 5.57% higher than that of "Sanditi" and 1.57% higher than that of all the control varieties. The contents of asparagine, threonine, serine, glutamic acid, valine, and histidine were higher in ZT1 than in the control varieties (Table 6).

The total contents of Mn and Fe in ZT1 were 4.65 and 39.97%, respectively, which were both higher than those in the control varieties ($P < 0.01$), by 23.3 and 23.2%, respectively (Table 7). The total content of Zn and Mg in ZT1 were 15.59 and 0.60%, respectively, which were both higher than those in the control varieties ($P < 0.01$), by 8.6 and 7.64%, respectively. In addition, the content of B in ZT1 was 5.15%, which was higher than that in the control varieties by 14.4% ($P < 0.01$). However, the content of Cu in ZT1 (1.57%) was not significantly different from that in the control varieties.

VMFR was significantly positively correlated with hay yield, CP content, and the total content of 18 essential AAs, as well as with the total content of Mn ($P < 0.05$; Table 8). VMFR was also significantly positively correlated with the total content of Zn ($P < 0.01$), and a weak positive correlation was observed between MFR and CF, total Fe content, and total Mg content ($P > 0.05$). In addition, MFR also had a weak negative correlation with seed yield and total Cu content ($P > 0.05$).

Discussion

Space mutation breeding is a novel breeding technology that has become popular in China over the past decade, since it combines advanced space techniques with conventional breeding (Sun *et al.,* 2017). Extensive research on the application of space mutation breeding in crop species, vegetables, and flowers has been conducted, and many varieties have been released for commercial production (Wang *et al.,* 2017). Previous studies have shown that space mutation breeding can potentially increase biomass, enhance stress resistance, improve quality, and induce early ripening, traits that are important for alfalfa improvement (Cyranoski, 2001; Norrmile and Ding, 2002; Li *et al.,* 2017; Li *et al.,* 2018).

Unlike other grain crops, the economic yield of alfalfa is mainly measured as the yield and quality of hay, which is composed of leaves and stems. Alfalfa leaves contain 60% of total digestible nutrients, 70% of the CP, and 90% of vitamins in the plant, so elite alfalfa varieties should be multifoliate with at least four leaflets per leaf (Wang, 2010; Yang *et al.,* 2013). The present study indicated that ZT1 had a BMFR of 83.49%, CMFR of 41.5% and VMFR of 34.64%, which were significantly higher than those of the multifoliate alfalfa variety "Concett" that was introduced from Canada and developed with conventional breeding

Table 1: Leaf and branch characteristics of "Zhongtian No. 1" (ZT1) alfalfa, compared to those of "Sanditi," "Zhonglan No. 1," "Concett," and "Longdong" across three years (2011–2013) at two locations (Lanzhou and Gangu) on the Loess Plateau, China

Location	Variety	BMFR (%)	LMFR (%)	VMFR (%)	L/B	D/W
Lanzhou	ZT1	82.53[aA]	41.50[aA]	34.65[aA]	1.014[aA]	0.21[a]
	Sanditi	0	0	0	0.922[bA]	0.23[a]
	Zhonglan No. 1	0	0	0	0.911[bA]	0.22[a]
	Concett	30.58[bB]	10.70[bB]	3.27[bB]	0.896[bA]	0.22[a]
	Longdong	-	-	-	-	-
Gangu	ZT1	84.31	43.02	36.27	1.034 [aA]	0.20[bB]
	Sanditi	0	0	0	0.927 [bA]	0.23[bB]
	Zhonglan No. 1	0	0	0	0.919 [bA]	0.21[bB]
	Concett	-	-	-	-	-
	Longdong	0	0	0	0.890 [bA]	0.28[aA]

Note: BMFR, branch multifoliate rate; LMFR, leaf multifoliate rate; VMFR, variety multifoliate rate; L/B, ratio of leaf number to branch number; D/W, ratio of dry weight to wet weight. Different lowercase letters in the same column indicate significant differences ($P < 0.05$), and different uppercase letters in the same column indicate very significant differences ($P < 0.01$)

Table 2: Plant height (PH) and hay yield (HY) of "Zhongtian No. 1" (ZT1) alfalfa, compared to "Sanditi," "Zhonglan No. 1," and "Concett" across three years (2011–2013) in Lanzhou, China

Year	Variety	First harvest		Second harvest		Third harvest		Total yield
		PH (cm)	HY (kg·ha^{-1})	PH (cm)	HY (kg·ha^{-1})	PH (cm)	HY (kg·ha^{-1})	HY (kg·ha^{-1})
2011	ZT1	74.9	5720.4	73.6	2860.2	46.7	2012.8	10,593.4
	Sanditi	71.2	4684.7	70.1	2342.3	45.5	1648.3	8675.3
	Zhonglan No. 1	75.5	5496.2	70.5	2748.1	39.1	1933.8	1,0178.1
	Concett	69.2	4399.3	68.0	2199.6	31.3	1547.9	8146.8
2012	ZT1	96.0	7301.7	92.1	4867.8	75.3	3042.3	15,211.8
	Sanditi	85.4	6562.8	83.5	4375.2	68.6	2734.6	13,672.6
	Zhonglan No. 1	94.7	7139.3	90.7	4759.6	72.2	2974.7	14,873.6
	Concett	73.3	6326.1	71.6	4217.4	60.7	2635.8	13,179.3
2013	ZT1	98.3	7946.4	97.1	5579.4	79.3	3381.5	16,907.3
	Sanditi	86.5	6990.5	84.5	4908.2	71.5	2974.7	14,873.4
	Zhonglan No. 1	96.7	7097.7	93.7	4983.5	76.2	3020.3	15,101.5
	Concett	76.2	6782.5	74.6	4762.2	63.9	2886.2	14,430.9
Mean	ZT1	89.7	6989.5	87.6	4435.8	67.1	2812.2	14,237.5
	Sanditi	81.0	6079.3	79.4	3875.2	61.9	2452.5	12,407.1
	Zhonglan No. 1	88.9	6577.7	84.9	4163.7	62.5	2642.9	13,384.4
	Concett	72.9	5836.0	71.4	3726.4	51.9	2356.6	11,919.0

Note: Different lowercase letters in the same column indicate significant differences ($P < 0.05$) and different uppercase letters of the same column indicate very significant differences ($P < 0.01$)

Table 3: Plant height (PH) and hay yield (HY) of "Zhongtian No. 1" (ZT1) alfalfa, compared to those of "Sanditi," "Zhonglan No. 1," and "Longdong" across three years (2011–2013) in Gangu, China

Year	Variety	First harvest		Second harvest		Third harvest		Total yield
		PH (cm)	HY (kg·ha^{-1})	PH (cm)	HY (kg·ha^{-1})	PH (cm)	HY (kg·ha^{-1})	HY (kg·ha^{-1})
2011	ZT1	76.3	5883.5	75.3	3922.3	/	/	12,257.2
	Sanditi	74.1	4886.1	72.1	3257.4	/	/	10,179.3
	Zhonglan No. 1	73.5	5377.9	72.7	3585.3	/	/	11,204.1
	Longdong	63.4	4895.4	70.0	3263.6	/	/	10,198.7
2012	ZT1	103.1	7471.9	95.6	5102.8	65.3	2369.2	18,224.3
	Sanditi	95.3	6594.1	87.3	4503.3	58.6	2090.8	16,083.2
	Zhonglan No. 1	94.7	7071.5	93.7	4829.3	52.2	2242.2	17,247.6
	Longdong	86.1	6620.4	75.1	4521.2	50.7	2099.2	16,147.3
2013	ZT1	108.1	8538.3	96.7	5559.8	68.1	2382.7	19,856.4
	Sanditi	96.5	7356.3	84.5	4790.1	62.7	2052.9	17,107.6
	Zhonglan No. 1	106.6	8097.7	92.3	5272.9	66.9	2259.9	18,831.9
	Longdong	89.2	7559.0	75.3	4922.1	58.4	2109.6	17,579.1
Mean	ZT1	99.2	7297.9	89.2	4861.6	66.7	2375.9	16,779.3
	Sanditi	91.9	6278.8	81.3	4183.6	60.6	2071.9	14,456.7
	Zhonglan No. 1	94.9	6849.0	86.2	4562.5	59.6	2251.1	15,761.2
	Longdong	82.2	6358.3	73.5	4235.6	54.6	2104.4	14,641.7

techniques. Whether mutation phenomenon of the high multifoliate ratio of ZT1 exists in other plants is unclear, and further work needs to be done in other plants.

In ZT1, the multifoliate trait increased the leaf mass by 5.72%, compared to that of the control varieties, and the increased leaf mass enhanced photosynthesis and subsequently plant growth. Therefore, the hay yield of ZT1 was significantly higher than that of the control varieties.

Table 4: Seed yield of "Zhongtian No. 1" (ZT1) alfalfa, compared to that of "Sanditi," "Zhonglan No. 1," "Concett," and "Longdong' Longdong" across three years (2011–2013) at two locations (Lanzhou and Gangu) on the Loess Plateau, China

Location	Variety	Seed yield ($kg \cdot ha^{-1}$)			
		2011	2012	2013	Mean
Lanzhou	ZT1	202.5	328.7	337.6	289.6^{bA}
	Sanditi	201.7	327.3	337.3	288.8^{bA}
	Zhonglan No. 1	207.5	339.4	347.4	298.1^{aA}
	Concett	184.6	303.7	303.8	265.6^{cB}
	Longdong	-	-	-	-
Gangu	ZT1	193.4	289.6	303.6	262.2^{bB}
	Sanditi	197.7	290.4	317.7	268.6^{bB}
	Zhonglan No. 1	212.4	311.8	334.4	286.2^{aA}
	Concett	-	-	-	-
	Longdong	183.5	265.1	290.0	246.2^{cC}

Note: Different lowercase letters of the same column indicate significant differences ($P < 0.05$), and different uppercase letters of the same column indicate highly significant differences ($P < 0.01$)

Table 5: Nutrient contents (%) of "Zhongtian No. 1" (ZT1) alfalfa, compared to that of "Sanditi," "Zhonglan No. 1," and "Concett" during the first and second harvests of 2012

Harvest	Variety	CP	CF	EE	Ash	Ca	P	NFE	Wa
First	ZT1	20.08 ± 0.05^{aA}	27.49	3.11	10.15	1.62	0.40	29.74	9.43
	Sanditi	19.72 ± 0.04^{abAB}	22.23	1.70	9.90	1.37	0.31	37.24	9.21
	Zhonglan No. 1	18.89 ± 0.06^{bcBC}	23.37	2.09	9.61	1.39	0.31	36.85	9.16
	Concett	19.90 ± 0.09^{aA}	18.83	3.12	10.72	1.55	0.34	37.03	10.4
Second	ZT1	18.42 ± 0.15^{aA}	22.78	1.68	9.31	0.87	0.53	37.99	9.82
	Sanditi	16.89 ± 0.13^{bB}	26.00	1.75	8.91	0.76	0.36	38.02	8.43
	Zhonglan No. 1	17.22 ± 0.09^{bB}	26.31	1.76	9.42	0.84	0.45	36.29	9.00
	Concett	18.18 ± 0.11^{aA}	23.92	1.94	9.16	0.84	0.43	37.49	9.31

Note: CP, crude protein; CF, crude fiber; EE, ether extract, Ash, ash content; Ca, calcium; P, phosphorus; NFE, nitrogen-free extract; Wa, water. Different lowercase letters in the same column indicate significant differences ($P < 0.05$), and different uppercase letters in the same column indicate highly significant differences ($P < 0.01$)

Table 6: Content of 18 essential amino acids of "Zhongtian No. 1" (ZT1) alfalfa, compared to "Sanditi," "Zhonglan No. 1," and "Concett" at anthesis in 2013

AAs	Varieties			
	ZT1	Sanditi	Zhonglan No. 1	Concett
Asn	2.17	2.00	2.04	2.00
Thr	0.56	0.53	0.57	0.54
Ser	0.60	0.56	0.58	0.56
Glu	1.26	1.16	1.30	1.24
Pro	1.22	1.22	1.32	1.33
Gly	0.56	0.52	0.56	0.58
Ala	0.61	0.56	0.62	0.60
Cys	0.26	0.26	0.28	0.24
Val	0.74	0.68	0.73	0.70
Met	0.18	0.20	0.18	0.18
Ile	0.50	0.48	0.53	0.50
Leu	0.78	0.73	0.82	0.79
Tyr	0.30	0.33	0.37	0.35
Phe	0.70	0.69	0.74	0.71
Lys	0.80	0.74	0.80	0.80
His	0.32	0.30	0.31	0.31
Arg	0.50	0.45	0.51	0.50
Trp	0.26	0.26	0.26	0.26
Total	12.32^{aAB}	11.67^{bB}	12.52^{aA}	12.19^{aAB}

AAs: amino acid

Note: Different lowercase letters in the same row indicate significant differences ($P < 0.05$), and different uppercase letters in the same row indicate highly significant differences ($P < 0.01$)

Increased leaf mass also contributed significantly to nutrient, AA, and trace mineral contents. However, although the results of the present study were quite positive, the relationships between varying levels of MFR indices and the yield and quality of alfalfa need to further investigated.

Correlation analysis indicated that VMFR in ZT1 was positively correlated with hay yield, CP content, AA content, and total Mn content at $P < 0.05$ and with total Zn content at $P < 0.01$. Correlation data indicated that the multifoliate trait increased leaf mass and effectively improved the hay yield and nutrient value of ZT1. However, VMFR was negatively correlated with seed yield, probably

Table 7: Total content (%) of six trace elements in "Zhongtian No. 1" (ZT1) alfalfa, compared to "Sanditi," "Zhonglan No. 1," and "Concett" at the anthesis stage of the first harvest in 2012

Nutrient	Variety			
	ZT1	Sanditi	Zhonglan	Concett
Zn	15.59 ± 0.18^{aA}	14.46 ± 0.16^{bBC}	14.57 ± 0.10^{bB}	14.01 ± 0.22^{cC}
Mn	4.65 ± 0.69^{aA}	4.01 ± 0.87^{bB}	4.13 ± 0.36^{bB}	3.20 ± 0.89^{cC}
Cu	1.57 ± 0.05^{bB}	1.71 ± 0.03^{aA}	1.58 ± 0.03^{aB}	1.43 ± 0.03^{cC}
Fe	39.97 ± 0.76^{aA}	39.87 ± 0.71^{aB}	30.15 ± 0.34^{bB}	26.72 ± 0.52^{cC}
Mg	0.60 ± 0.03^{aA}	0.59 ± 0.02^{aA}	0.59 ± 0.02^{aA}	0.52 ± 0.04^{bA}
B	5.15 ± 0.04^{aA}	4.55 ± 0.04^{cC}	4.10 ± 0.06^{dD}	4.90 ± 0.05^{bB}

Note: Different lowercase letters in the same column indicate significant differences ($P < 0.05$), and different uppercase letters in the same column indicate very significant differences ($P < 0.01$)

Table 8: Correlation coefficients of 11 quantitative and qualitative parameters in "Zhongtian No. 1" (ZT1) alfalfa

Index	VMFR	HY	SY	CP	CF	AA	Zn	Mn	Cu	Fe
HY	0.656*									
SY	-0.058	0.674*								
CP	0.601*	-0.006	-0.406							
CF	0.676	0.956**	0.678*	0.116						
AA	0.237*	0.496	0.309	-0.142	0.275					
Zn	0.790**	0.925**	0.524	0.270	0.961**	0.317				
Mn	0.522*	0.915**	0.791**	0.020	0.977**	0.202	0.902**			
Cu	-0.232	0.221	0.715**	-0.089	0.398	-0.316	0.285	0.559		
Fe	0.401	0.488	0.488	0.370	0.706*	-0.349	0.685*	0.758**	0.757	
Mg	0.166	0.623*	0.790**	0.037	0.712**	0.165	0.606*	0.786**	0.710	0.649*

Note: VMFR, variety multifoliate rate; MFR, multifoliate rate; HY, hay yield; SY, seed yield; CP, crude protein content; CF, crude fiber content; AA, amino acid content. The eleven parameters were analyzed using the $y = ax + b$ model
**indicates significance at $P < 0.01$ level
*indicates significance at $P < 0.05$ level

because of enhanced photosynthesis in plants and significantly improved biomass. The VMFR indices of ZT1 grown in Gangu (semi-humid experimental site) were also slightly higher than those grown in Lanzhou (semi-arid experimental site), so the effect of geographical location on VMFR indices needs further evaluation using multiple experimental sites.

In addition to field evaluation and selection, basic research will also be needed in the future to (1) comprehensively and systemically elucidate the hereditary characteristics of space mutations from multiple perspectives, such as morphology, physiological biochemistry, and molecular biology; (2) better understand the molecular regulatory mechanisms of space mutations, identifying the hot spots of induced mutations using molecular techniques; (3) investigate the epigenetic mechanisms of space mutations; (4) map and clone mutant genes through linkage mapping and develop molecular markers related to mutated phenotypes, which will promote molecular marker-assisted selection and further improve breeding effectiveness; (5) accelerate the development and release of new and improved varieties; and (6) further promote space mutation breeding for forage crops.

Conclusion

Zhongtian No. 1 is the first multifoliate alfalfa variety developed *via* space mutation breeding and conventional selection in China. "Zhongtian No. 1" alfalfa was characterized by high quality and high productivity, including high multifoliate rate, high yield potential, and high nutrient content. The plant exhibited an average of five leaflets per leaf, average CMFR of 41.5%, and DW/FW of 50.36%. Meanwhile, the hay yield was 15,508.4 kg·ha^{-1}, CP content was 20.08%, and AA content was 12.32%. Results demonstrated that the new alfalfa variety ZT1 exhibited good performance and quality, which had wide application prospect.

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