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RESEARCH ARTICLE

Kauke: A new alfalfa cultivar with high persistence and productivity in the Mediterranean drylands of Chile

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ABSTRACT

Mediterranean dryland environments are among the most threatened by climate change. The objective of this work was to report the agronomic value of 'Kauke', a new alfalfa (*Medicago sativa* L.) cultivar developed by INIA's Alfalfa Breeding Program for the Mediterranean drylands of Chile. 'Kauke' was developed using a within-family selection method from an interspecific hybrid between *M. sativa* and *M. arborea* L. Multi-location trials were established across five sites representing a gradient of dryland environments. Results showed that 'Kauke' exhibited a highly significant Genotype × Location interaction. In the most stressed environments (Cauquenes and Portezuelo), 'Kauke' produced 40% more forage yield than control cultivars (ACB650 and WL903). Furthermore, 'Kauke' demonstrated superior persistence, maintaining a plant density 40% higher than control cultivars after three growing seasons in drought-prone areas. Categorized as a semi-dormant type (Fall Dormancy 7), 'Kauke' represents a strategic breakthrough for adapting rainfed agriculture to new climatic scenarios in central-south Chile.

Key words: Drought tolerance, interspecific hybrid, *Medicago sativa*, persistence, rainfed agriculture.

INTRODUCTION

Mediterranean dryland environments are among the most threatened by climate change. Increases in temperature, along with declining rainfall and greater interannual variability, are affecting dryland farming systems and their profitability, due to reduced water availability for forage yield and therefore livestock production (del Pozo et al., 2017; Inostroza et al., 2021; Noushahi et al., 2025a; 2025b; Noushahi and del Pozo, 2026).

In Chilean Mediterranean dryland, livestock farming is a key productive component of systems, relying primarily on natural grasslands, which have relatively low productivity (2-4 t DM ha^{-1} yr^{-1}) (Ovalle et al., 2015). The sowing of annual pastures, for grazing and conservation, has been a successful strategy to intensify livestock systems. However, rising cost of agricultural inputs and limited access to machinery for soil preparation and sowing have led to a decrease in the practice of sowing annual pastures. In addition, the Chilean forage seed market is based on imported seeds, which generates uncertainty every year regarding seed availability and price (Moscoso et al., 2021).

In this context, the Instituto de Investigaciones Agropecuarias (INIA) has investigated the use of perennial forage legumes, such as alfalfa (*Medicago sativa* L.), as a strategy to enhance the adaptation of Mediterranean dryland livestock systems to increasingly severe drought conditions. Alfalfa is a perennial forage species well known for its natural tolerance to drought, largely due to its deep and extensive root system, which allows access to water stored in deeper soil layers. It is considered one of the oldest cultivated forage crops in human history, valued for its high nutritional quality, productivity, and persistence under a wide range of environmental conditions. Originally domesticated in Central Asia, it was introduced to South America by the Spanish settler during the conquest. It was primarily used to feed horses and also recognized for its medicinal properties, including its use in treating wounds. Chronicles recount that it entered through Peru and immediately spread to northern Chile, where it has been cultivated for over 400 yr in the desert and altiplano valleys (Inostroza et al., 2021).

In Chile, there are no varieties in the Agricultural and Livestock Service's (SAG) Register of Varieties Suitable for Certification (RVAC); however, there are 60 varieties in the List of Officially Described Varieties (LVOD). Most are high-yielding cultivars developed by leading genetic companies worldwide. These cultivars have undergone extensive genetic improvement, with a strong emphasis on maximizing productivity under irrigated conditions, often at the expense of traits related to environmental adaptation and resilience. Genetic studies indicate that during domestication and genetic improvement, modern alfalfa cultivars have lost nearly 30% of their genetic diversity. This loss is associated with a reduced capacity to adapt to unfavorable environments (Humphries et al., 2020; Inostroza et al., 2021).

Conversely, in other temperate regions such as Australia and Argentina, alfalfa is grown primarily under dryland conditions by utilizing its natural attributes—specifically, the high drought tolerance conferred by its deep root system (Humphries et al., 2020). To successfully adapt alfalfa to Mediterranean dryland conditions of central-south Chile, it is fundamental to develop new cultivars that are drought-tolerant and have greater water-use efficiency (Humphries et al., 2020). These attributes are essential for productivity and persistence in environments where the plant must survive 4-6 mo of drought (del Pozo et al., 2017).

The INIA's Alfalfa Breeding Program (ABP) has transitioned through distinct methodological eras. The initial phase focused on evaluating the agronomic value of foreign cultivars, leading to the introduction of high-yielding materials (Avendaño et al., 1972; López et al., 1989; Romero et al., 1989). By the late 1970s and 1980s, the program shifted toward evaluating introduced landraces—mainly from Europe—and performing recurrent phenotypic selection. This methodology allowed breeders to increase the frequency of favorable alleles for forage yield and nematode resistance. This era produced pivotal cultivars such as Rayén-INIA, Palihue-INIA, and Huinca-INIA, which redefined regional production standards materials (Avendaño et al., 1972; López et al., 1989; Romero et al., 1989). 'Rayén-INIA' was developed to exploit the high-potential irrigated lands of the Central Valley, utilizing non-dormant germplasm to maximize winter growth (winter activity). Following this, 'Palihue-INIA' was released in 1989 as a direct response to the devastating impact of stem nematodes in the Aconcagua and Maipo valleys. Furthermore, 'Palihue-INIA' demonstrated that domestic breeding could achieve higher persistence than leading international cultivars like CUF-101 through targeted selection for pest resistance. In contrast, 'Huinca-INIA' represented a strategic shift toward the cooler latitudes of the south-central region, incorporating a higher degree of winter dormancy to ensure frost survival and field longevity.

In recent years, the focus of INIA's ABP has evolved, transitioning from the high-input irrigated models of the 20th century toward climate-smart agriculture. In 2015, INIA introduced to Chile a collection of 70 alfalfa accessions originating from different semi-arid environments of the world (Humphries et al., 2020; Inostroza et al., 2021; Noushahi et al., 2025a; 2025b). The collection was provided by Crop Trust, an international organization dedicated to the conservation of the genetic diversity of cultivated plants. The collection included alfalfas from Afghanistan, Argentina, Australia, China, USA, Spain, India, Iran, Kazakhstan, Peru, Russia, and Türkiye. In addition, INIA included 12 alfalfa populations collected in the desert and altiplano valleys of Chile, which are known as 'Alta Sierra'. With this gene pool, INIA's ABP has resumed its genetic improvement work to develop drought-tolerant cultivars that offer high productivity, persistence, and forage quality for the Mediterranean drylands of Chile. The objective of this work was to report the agronomic value of 'Kauke', a new alfalfa cultivar developed by INIA's ABP for the Mediterranean drylands of Chile.

MATERIALS AND METHODS

Population development

The alfalfa (*Medicago sativa* L.) 'Kauke' population was developed using a within-family selection method (Casler and Vogel, 1999). It originated from the crossing of plants selected from the CTA002 family, provided to the Instituto de Investigaciones Agropecuarias (INIA) Alfalfa Breeding Program (ABP) by the Australian Pasture Genebank of the South Australian Research Development Institute (SARDI; https://apg.pir.sa.gov.au/gringlobal/search), within the framework of the project "The use of crop wild relatives to develop drought tolerant alfalfa and its extension to subsistence farmers in Kazakhstan, China and Chile (GS19001)".

Among 70 populations evaluated at the Cauquenes Experimental Station during the 2017-2018 and 2018-2019 growing seasons—characterized by a summer period exceeding 5 mo—the CTA002 population showed outstanding agronomic performance, achieving the highest forage production and persistence under rainfed conditions, and also performing well in Adelaide, Australia (Inostroza et al., 2021; del Pozo et al., 2023). The CTA002 population originates from the cross *M. sativa* 'Genesis' × 'Alborea101' (Irwin and Bingham, 2023). Genesis is a cultivar of Australian origin, released in 1996, widely adapted to Mediterranean climate conditions. 'Alborea101' corresponds to an interspecific hybrid between *M. sativa* ($2n = 4x = 32$) and *M. arborea* ($2n = 4x = 32$) developed by the University of Wisconsin, USA. Genetic studies have shown that 'Alborea101' has less than 5% of the *M. arborea* genome. The Alborea hybrid seeks to introgress traits conferring abiotic stress tolerance (drought and salinity) and larger seed size (Bingham et al., 2013; Irwin and Bingham, 2023).

In 2017, 750 plants of CTA002 were established at the Cauquenes Experimental Station of INIA, located in the city of Cauquenes, Maule Region (35°57' S; 72°19' W). The plants were grown under rainfed conditions. They were established in rows spaced 20 cm apart; within the row, plants were planted every 10 cm. During two growing seasons (2017-2018 and 2018-2019), DM production and plant persistence were evaluated after two summers with at least 5 mo without rain (Inostroza et al., 2021; del Pozo et al., 2023).

In the autumn of 2019, 85 plants of CTA002 were selected. Selection was based on plant vigor attributes after the summer (green stems, crown size, and erect growth). Three stems per plant were collected, including a crown section. The stems were wrapped in moistened absorbent paper and placed in sealed bags. They were immediately taken to the greenhouse for rooting. The stems were dipped in rooting hormone (naphthyl acetic acid 0.4%; Keri Root, Yates Ltda., Santiago, Chile) and planted in tubes 11 cm in diameter and 50 cm in height, containing a 50% sand and 50% peat moss v/v mixture. The first generation of the 'Kauke' population was obtained during the winter and spring of 2019. Flowering was induced with an 18:6 h photoperiod. Bombus (*Bombus terrestris*) was used as the pollinating agent (Natupol, Koppert, Quillota, Chile). The seed was harvested in bulk.

The second generation of 'Kauke' population (seed increase) was also obtained in the greenhouse. In February 2020, the seed was sown in germination trays and inoculated with a *Sinorhizobium meliloti* (strain WSM2141) suspension. When plants reached three plenty expanded leaves, they were transplanted into containers of 1 m³ capacity (1 × 1 × 1m). The plants were distributed at 20 cm between rows and 10 cm within the row. Ten square meters (10 cubes) were planted. During the winter and spring of 2020, the second generation of 'Kauke' population was obtained. Flowering was induced with an 18:6 h photoperiod supplied by 400-watt metal-halide lamps. Bombus was again used as the pollinating agent (Natupol). The seed was harvested in bulk, yielding 800 g. The genetic seed of the 'Kauke' population is maintained in the INIA genebank. The seed was increased from G0 to pre-basic by establishing a 2000 m² seed production stand in Los Ángeles, Biobío Region, Chile, isolated by distance.

Multi-location trials establishment and agronomical management

From April 2021 to June 2022, five field trials were established across the Maule (Cauquenes), Ñuble (Chillán, El Carmen, and Portezuelo), and La Araucanía (Vilcún) Regions. The sowing dates and geographic locations for each site are shown in Table 1. All trials were managed under rainfed conditions, except for Chillán, where the trial received irrigation to estimate yield potential under irrigated conditions. The sites encompass all dryland environments from sea level to the Andes foothill, with a summer season lasting from 3 to 6 mo (Table 1). In all locations, soil preparation was conducted through a sequence of chiseling, disc harrowing, and seedbed refinement using a rotary tiller. Fertilization was applied according to chemical soil analysis. Seven advanced genetic lines (AGLs) and two control cultivars (ACB650 and WL903) were established. The AGLs included: 'Kauke', SelAPG19018, SelAPG6567, SelAF3347, SelAS1, SelAS2, SelGen1. Each accession was sown in 2 × 4 m plots with a seeding rate of 15 kg ha^{-1}. Within the plots, seeds were distributed in rows spaced 20 cm apart. Each experiment was organized in a randomized complete block design with three replicates.

Following the establishment year, all experiments were fertilized in late winter 2022 and 2023 with 100 kg ha^{-1} Sulpomag (22% S, 22% K_2O, 18% MgO), 100 kg ha^{-1} potassium chloride (60% K_2O), and 100 kg ha^{-1} triple superphosphate (46% P_2O_5). Additionally, during the same period, chemical weed control was conducted by applying paraquat dichloride (5 L ha^{-1} in 200 L water; Gramoxone, Singenta, Greensboro, North Carolina, USA). At the beginning of 2021-2022, 2022-2023, and 2023-2024 growing seasons, all field trials were registered in the Registry of Agronomic Value Trials of the Seed Division of the Agricultural and Livestock Service (SAG).

Table 1. Sowing dates and environmental descriptors of multi-location trials for evaluating the agronomic value of advanced genetic lines of alfalfa.

Location	Annual rainfall	Altitude	Dryland region	Soil	Summer/dry season	Georeference	Sowing date
	mm	m a.s.l.			mo		
Cauquenes	518	135	Inner	Granitic	6	35°57.281' S 72°17.228' W	4 Jun 2021
Portezuelo	740	132	Inner	Granitic	5	36°28.468' S 72°27.703' W	7 May 2021
Chillán	908	140	Central valley	Andisol	5	36°31.715' S 71°54.741' W	21 Apr 2021
El Carmen	1218	400	Andes foothill	Andisol	4	36°54.710' S 71°54.036' W	1 Jun 2022
Vilcún	1114	325	Andes foothill	Andisol	3	38°41.557' S 72°24.898' W	21 Apr 2021

Agronomic traits

Forage yield (FY) was evaluated by harvesting with a cutter bar, leaving a 5 cm stubble height. A fresh biomass sample (300-400 g) was collected to determine DM content with a forced-air oven drying at 65 °C until a constant weight. Harvest dates for each location and growing season are presented in Table 2. The FY was added to estimate seasonal distribution (spring, summer, and autumn) and annual FY.

Table 2. Cutting dates across growing seasons in multi-location trials to evaluate the agronomic value of advanced genetic lines of alfalfa. Locations: Cauquenes, Portezuelo, Chillán, El Carmen, and Vilcún.

Growing season	Locations				
	Cauquenes	Portezuelo	Chillán	El Carmen	Vilcún
2021-2022	2 Feb 2022		20 Jan 2022	27 Jan 2022	2 Dec 2021
	5 Mar 2022		4 Mar 2022	6 Mar 2022	28 Jan 2022
	4 Apr 2022		5 Apr 2022	6 Apr 2022	4 Feb 2022
					4 Mar 2022
					5 Apr 2022
2022-2023	17 Oct 2022	16 Jan 2023	2 Sep 2022	1 Sep 2022	26 Dec 2022
	2 Nov 2022	7 Mar 2023	6 Nov 2022	28 Nov 2022	14 Feb 2023
	16 Jan 2023		17 Jan 2023	18 Jan 2023	
			9 Feb 2023		
2023-2024	3 Oct 2023	13 Oct 2023	21 Nov 2023	19 Oct 2023	3 Jan 2024
	5 Dec 2023	14 Dec 2023	8 Jan 2023	23 Nov 2023	14 Feb 2024
		5 Mar 2024	20 Feb 2024	23 Jan 2024	

The date of flowering was recorded at each location according to UPOV TG/6/5 guidelines (UPOV, 2005), this corresponds to the date when at least three inflorescences per plot were observed. Measurements were performed in the second growing season, starting 21 September 2022. Plots were evaluated twice a week (Mondays and Fridays). At full bloom, the length of the longest stem, including the inflorescence, was measured in 10 plants per plot.

At the beginning (spring) and end (autumn) of each growing season, plant persistence was evaluated in each experimental unit. For this purpose, 1 m linear section was selected from the central third of each plot. A measuring tape was used to mark the linear meter, and the number of plants was counted. This data was used to calculate the number of plants per square meter.

Fall dormancy experiment

Fall dormancy (FD) was estimated according to the methodology proposed by UPOV TG/6/5 guidelines (UPOV, 2005). The experiment was located at INIA Quilamapu research station in Chillán (36°35.676' S, 72°5.331' W), Chile. In the winter of 2022, seven alfalfa cultivars with known fall dormancy category (FD values 1 to 10) plus the seven AGLs were sown in germination trays. The control cultivars were: Aohan (FD = 1), Ameristand-201 (FD = 2), Osimtal (FD = 3), ACB350 (FD = 4), SARDI5(FD = 5), ACB550 (FD = 8), SARDI10 (FD = 10). In October 2022, plants were transplanted to the field in single-row plots, three m in length. Rows were spaced 0.75 m apart. Within each row, plants were established every 30 cm (10 plants per row). The experiment was organized in a randomized complete block design with three replicates. Prior to planting, the experimental area was fertilized with the equivalent of 200 kg ha^{-1} triple superphosphate and 100 kg ha^{-1} potassium chloride. The trial was managed under irrigated conditions. All plots were mowed 2 wk before the autumn equinox (3 March 2023) using a cutter bar, leaving 5 cm stubble height. Plant height of seven plants per plot was measured at the autumn equinox (21 March 2023) and then, on 5 April, 2 May, and 1 June 2023. The relationship between plant height and the FD of varieties with known dormancy values was fitted to a linear model using R software (R Foundation for Statistical Computing, Vienna, Austria). A linear model was adjusted for each sampling date, and its coefficient of determination (R^2) was determined. The FD of the candidate lines was predicted using the mean value of the three fitted models.

Statistical analysis

Variables were analyzed with ANOVA at a 95% confidence level. Means were compared using the least significant difference (LSD) test with the agricolae package in R software (De Mendiburu, 2023). Forage yield was fitted to a mixed model using the restricted maximum likelihood (REML) method in ASReml-R software (Butler, 2020). Populations (P), locations (L), and the P×L interaction were considered as fixed effects, while replicates were considered as random effects. The P×L interaction was interpreted through principal component analysis (PCA) using the GGEbiplot package in R (Frutos et al., 2014).

RESULTS

Forage yield

The combined ANOVA for FY across locations, revealed a highly significant P×L interaction ($P < 0.001$; Table 3). The P × L interaction was significant in each growing season. The interaction pattern remained consistent across growing seasons; therefore, the average of the final two growing seasons was analyzed. The PCA analysis showed that the locations were grouped into three mega-environments (ME): ME1, which included Cauquenes and Portezuelo; ME2, which included Vilcún and Chillán; and ME3, which included El Carmen (Figure 1). The 'Kauke' population exhibited the highest FY (7000 kg DM ha^{-1}) in Cauquenes and Portezuelo (ME1; Figure 1), the most extreme environments characterized by 500-700 mm annual rainfall, low-fertility granitic soils, and 5-6 mo of drought (Table 1 and Figure 1). Under these conditions, 'Kauke' produced 40% more forage yield than the control cultivars (ACB650 and WL903; Table 4). In Chillán and Vilcún (ME2), 'Kauke' had similar FY than the control cultivars ($P > 0.05$; Table 4). In Chillán, under irrigated conditions, 'Kauke' produced 19 000 kg DM ha^{-1}, mirroring the high yield potential of the variety under favorable water conditions. In Vilcún, annual FY was 4000 kg DM ha^{-1}, which was low for an environment with 1200 mm annual precipitation. This was due to very cold and rainy springs during the second (2022-2023) and third (2023-2024) growing seasons. At the El Carmen (ME3), 'Kauke' achieved a FY of 7700 kg ha^{-1}, which was statistically similar to that of control cultivars (Table 4).

Table 3. ANOVA and F-values for effects of Location (L), Population (P), and their interaction (L × P) on alfalfa 'Kauke' forage yield, evaluated across five locations (Cauquenes, Portezuelo, Chillán, El Carmen, and Vilcún) and three growing seasons (2021-2022, 2022-2023, and 2023-2024). **, and *** for $P < 0.01$, and $P < 0.001$, respectively.

Variables	Location (L)	Population (P)	L × P
Forage yield season 2 (2022/2023)	223.4***	4.6***	4.0***
Forage yield season 3 (2023/2024)	321.4***	5.5***	2.20**
Forage yield across seasons 2 and 3 (2022/2023 and 2023/2024)	580.7***	4.6***	2.8***

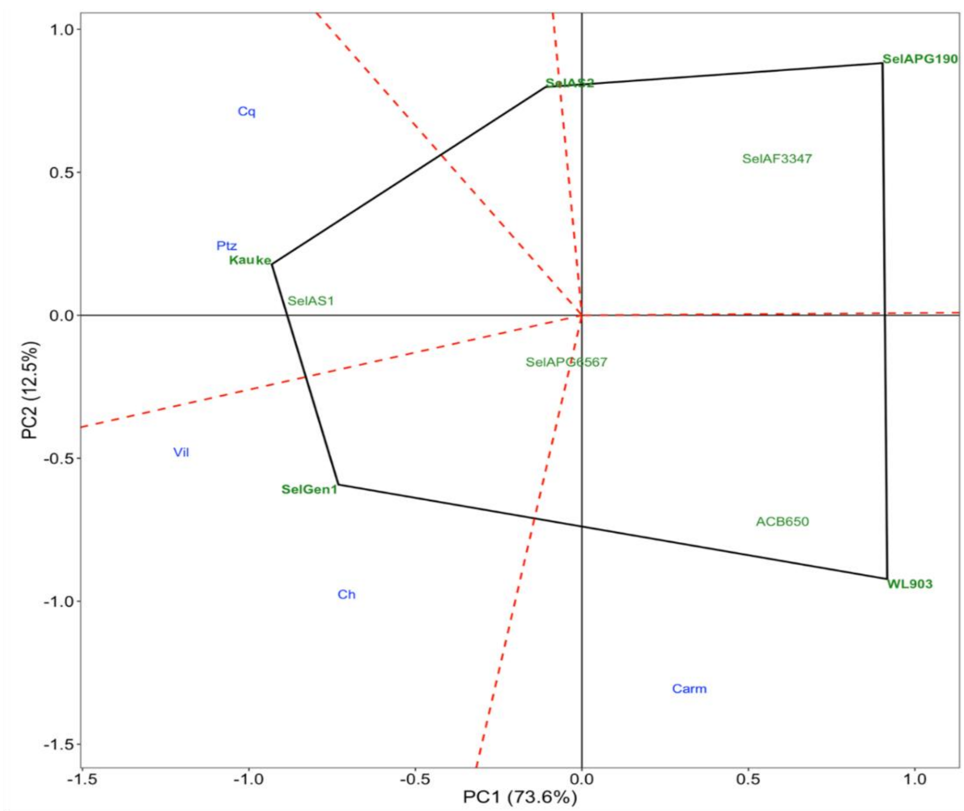

Figure 1. GGE biplot for the forage yield (FY) of seven advanced genetic lines of alfalfa ('Kauke', SelAS2, SelAPG190, SelAF3347, SelAS1, SelAPG6567, SelGen1) and two control cultivars (ACB650 and WL903), evaluated across five locations: Cauquenes (Cq), Portezuelo (Ptz), Chillán (Ch), El Carmen (Carm), and Vilcún (Vil). The GGE biplot was constructed using the first two principal components (PC1 and PC2) from the principal component analysis of FY across 5 locations and 9 alfalfa populations.

Table 4. Annual forage yield of alfalfa ‘Kauke’ and control cultivars (ACB650 and WL903) evaluated in multi-location trials over three growing seasons. *, **, and *** for $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; ^{ns}nonsignificant; CV: coefficient of variation.

		Growing seasons			
Locations	Populations	2021-2022	2022-2023	2023-2024	Mean
		kg ha^{-1}			kg ha^{-1}
Cauquenes	Kauke	1658^{ab}	6441^{ab}	8866^{ab}	7653^{a}
	ACB650	718^{d}	4891^{cd}	6558^{bc}	5724^{bc}
	WL903	493^{d}	4896^{cd}	5273^{c}	5084^{c}
	Population mean (n = 9)	1136	5419	7748	6583
	Significance	***	*	*	*
	CV, %	27.3	14.9	19.0	16.0
Portezuelo	Kauke		5693^{ab}	7125^{a}	6409^{a}
	ACB650		4469^{cd}	5425de	4947^{bc}
	WL903		3572^{d}	4820^{e}	4196^{c}
	Population mean (n = 9)		5025	6035	5530
	Significance		**	**	**
	CV, %		1.6	10.4	10.5
Chillán	Kauke	5894	19 770^{ab}	18 326^{abc}	19 048^{a}
	ACB650	4827	20 799^{a}	16 486^{cd}	18 642^{a}
	WL903	5360	21 609^{a}	16 732^{bcd}	19 170^{a}
	Population mean (n = 9)	4918	18 831	17 424	18 128
	Significance	ns	*	*	*
	CV, %	16.0	12.5	6.8	7.8
Vilcún	Kauke	4204^{b}	4487	4130	4309
	ACB650	7591^{a}	4808	3191	3999
	WL903	4503^{b}	4984	2890	3937
	Population mean (n = 9)	3801	5007	3182	4095
	Significance	***	ns	ns	ns
	CV, %	28.9	16.4	23.3	16.3
El Carmen	Kauke	468^{abc}	7740^{ab}	7803^{bc}	7772^{abc}
	ACB650	519^{ab}	9420^{a}	9355^{ab}	9388^{a}
	WL903	586^{a}	8880^{a}	9133^{ab}	9006^{ab}
	Population mean (n = 9)	426	7402	7712	7557
	Significance	*	*	**	***
	CV, %	18.3	16.5	16.5	13.6

Annual forage distribution

Harvests were aggregated by season into three categories: Spring, summer, and autumn. In Cauquenes and Portezuelo (ME1), 80% of forage production was obtained in spring, with the remaining 20% in January. ‘Kauke’ exhibited the same forage production distribution pattern as the control cultivars (Figure 2). In Chillán and Vilcún (ME2), two distribution patterns were observed, determined by irrigation status. In Chillán, under irrigated conditions, 50% of the annual forage production occurred in autumn (25%) and spring (25%), while the remaining 50% occurred in summer. In Vilcún, 50% of the annual forage production occurred in spring and the remaining 50% in summer. In El Carmen (ME3), 50% of the annual forage production occurred in spring, 40% in summer, and 10% in autumn. In all MEs, ‘Kauke’ exhibited the same FY distribution pattern as the control cultivars (Figure 2).

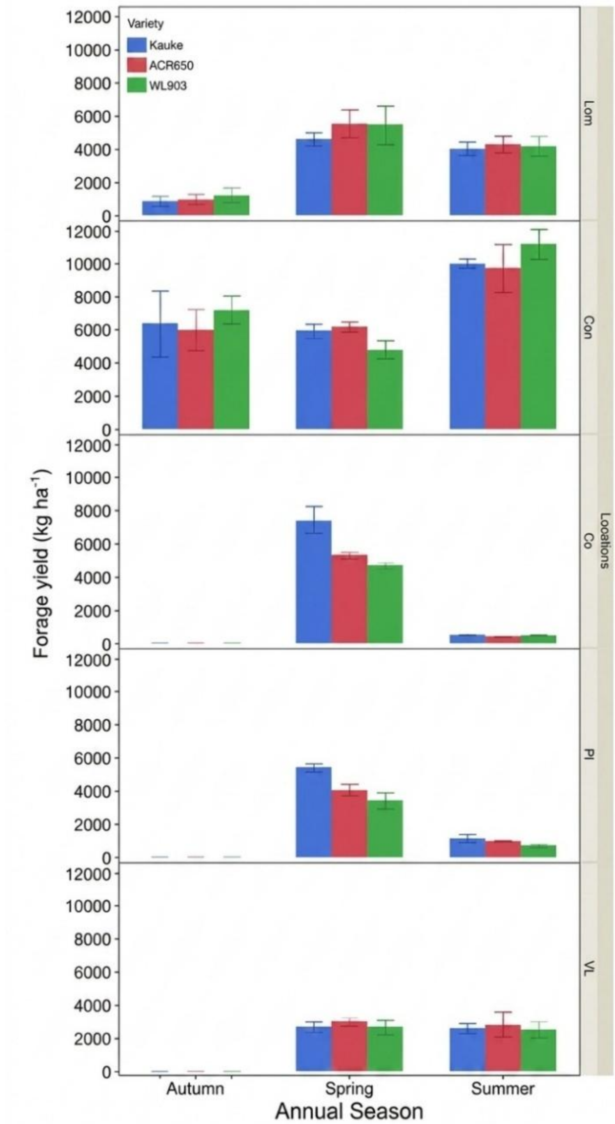

Figure 2. Seasonal distribution of annual DM yield during autumn, spring, and summer for the alfalfa 'Kauke' population and two control cultivars (ACB650 and WL903), evaluated across five locations: Cauquenes (Cq), Portezuelo (Ptz), Chillán (Ch), El Carmen (Carm), and Vilcún (Vil). Values represent the mean of two growing seasons (2022-2023 and 2023-2024). Error bars indicate the standard error of the mean.

Fall dormancy and flowering

The relationship between plant height and FD for cultivars with known dormancy was analyzed using a linear model. Three equations were derived with a coefficient of determination (R^2) greater than 0.8, indicating a strong fit (Figure 3). These equations were used to estimate the FD of the seven alfalfa AGLs. The mean FD value from these three equations was used to assign an FD category to each line. Based on this analysis, 'Kauke' achieved an FD of seven, placing it in the semi-dormant category, which is similar to ACB650 (FD = 6).

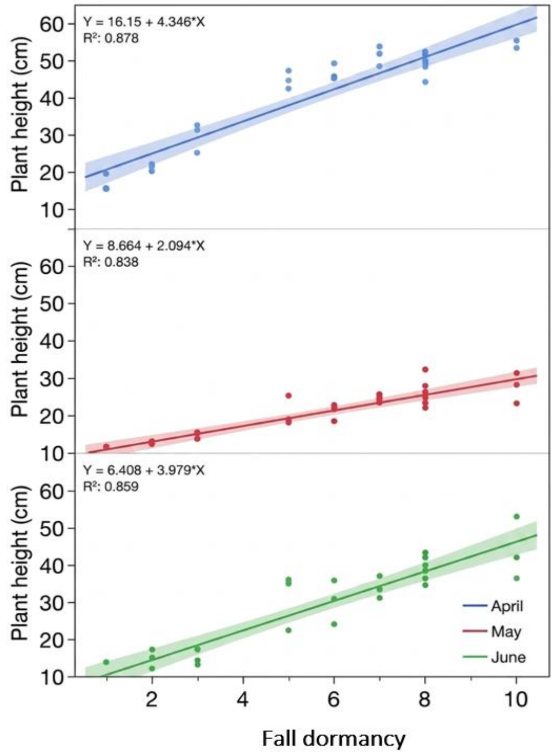

Figure 3. Linear regression of the relationship between fall dormancy (FD) of control alfalfa cultivars (known FD score) and plant height, fitted at 16 (April), 43 (May), and 72 (June) d after the first autumn equinox following sowing (21 March 2023).

Flowering dates were evaluated at four locations (Cauquenes, Chillán, El Carmen, and Vilcún) and results are presented as the average of two growing seasons. Flowering date varied markedly among locations, occurring at 37, 54, 60, and 60 d after the spring equinox (DAE; 21 September) in Cauquenes, Chillán, El Carmen, and Vilcún, respectively (Table 5). Significant differences among varieties were observed at all sites except Chillán. 'Kauke' showed similar flowering date to the control ACB650, reaching first bloom at 40, 55, 60, and 60 DAE in Cauquenes, Chillán, El Carmen, and Vilcún, respectively. 'Kauke' produces pale purple flowers, with no presence of variegated, yellow, or white flowers.

Table 5. Date of flowering, expressed as number of days after spring equinox (September 21; DAE), for the alfalfa 'Kauke' population and two control cultivars (ACB650 and WL903). Data were recorded across four locations (Cauquenes, Chillán, El Carmen, and Vilcún) and correspond to the mean of the 2021-2022 and 2022-2023 growing seasons. $^*P < 0.05$; ^{ns}nonsignificant; CV: coefficient of variation.

	Date of flowering (DAE)			
Populations	Cauquenes	Chillán	El Carmen	Vilcún
Kauke	40^{ab}	54	60^{ab}	57^{cd}
ACB650	36^{bc}	54	64^{a}	63^{abc}
WL903	33^{bc}	57	60^{ab}	60^{bcd}
Population mean (n = 9)	37	54	60	60
Significance	*	ns	*	*
CV, %	15.0	7.2	5.6	5.2

Pasture persistence

Plant density showed nonsignificant differences among varieties during the establishment year (50-60 plants m^{-2}). Differences between varieties were observed from the second season onward (Table 6). In Cauquenes, plant density decreased by 25% and 40% relative to the establishment year in the second and third growing seasons, respectively. In Portezuelo, plant density decreased by 60% relative to the establishment year in the second growing season. In Cauquenes and Portezuelo, 'Kauke' showed significantly higher plant density than control cultivars; it exceeded controls by 40% in Cauquenes (third growing season) and by 22% in Portezuelo (second growing season). In Chillán, plant density decreased by 20% and 25% in the second and third seasons, respectively. In El Carmen, the plant density decreased by 28% and 38%, and in Vilcún, it decreased by 46% and 56% during the second and third growing seasons, respectively. In Chillán and El Carmen, 'Kauke' maintained a plant density statistically equal to control cultivars (ACB650 and WL903; $P > 0.05$) across all growing seasons (Table 6). In Vilcún, 'Kauke' also maintained a 40% higher plant density by the end of the third growing season (Table 6).

Table 6. Plant density of the alfalfa 'Kauke' population and two control cultivars (ACB650 and WL903), evaluated during the establishment year (2021-2022) and at the end of the 2022-2023 and 2023-2024 growing seasons in multi-location trail including five locations. *, **, and *** for $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; ^{ns}nonsignificant; CV: coefficient of variation.

Location	Populations	Growing seasons		
		2021-2022	2022-2023	2023-2024
		plants m^{-2}		
Cauquenes	Kauke	44.3	32.3^{c}	33.0^{a}
	ACB650	44.3	29.6^{cd}	16.7^{b}
	WL903	44.0	23.6^{d}	17.0^{b}
	Populations mean (*n* = 9)	48.6	36.3	28.5
	Significance	ns	***	**
	CV, %	12.7	11.8	18.6
Portezuelo	Kauke		98.3	48.3^{a}
	ACB650		96.0	35.0^{bc}
	WL903		113.0	40.0^{abc}
	Populations mean (*n* = 9)		95.4	40.6
	Significance		ns	*
	CV, %		15.1	12.7
Chillán	Kauke	57.6	45.3	43.3
	ACB650	54.0	45.6	43.3
	WL903	57.0	50.6	41.6
	Populations mean (*n* = 9)	53.5	42.6	40.2
	Significance	ns	ns	ns
	CV, %	11.3	12.7	12.3
Vilcún	Kauke	60.6^{bc}	40.7^{a}	39.3^{a}
	ACB650	62.7^{b}	23.3^{d}	22.6^{b}
	WL903	61.0^{bc}	35.0^{b}	26.0^{b}
	Populations mean (*n* = 9)	61.5	33.0	26.5
	Significance	**	***	***
	CV, %	5.7	8.3	10.7
El Carmen	Kauke	54.3	48.3	33.3
	ACB650	47.3	35.0	30.0
	WL903	46.3	43.3	35.0
	Populations mean (*n* = 9)	56.8	40.9	35.2
	Significance	ns	ns	ns
	CV, %	13.6	15.4	11.9

DISCUSSION

Rainfed agriculture in Central Chile has endured a persistent “megadrought” since 2010, with annual rainfall deficits ranging from 20% to 40% (Garreaud et al., 2020). In this context, the development and release of the alfalfa ‘Kauke’ represents a strategic breakthrough for adapting rainfed agriculture to new climatic scenarios. As the first alfalfa cultivar specifically developed and released in Chile for rainfed environments, ‘Kauke’ addresses the limitations of traditional forage systems based on natural grasslands or improved pastures dominated by annual legumes. In the case of annual legumes, they typically concentrate their biomass production in almost a single month of the year and senesce early in the season due to water deficit. Furthermore, the biomass production depends on a high amount of P fertilizer, and commercial seed for annual sowing originates overseas, causing periodic uncertainty regarding seed availability and price (Moscoso et al., 2021).

‘Kauke’ produced 40% more forage yield (FY) than the standard control cultivars, ACB650 and WL903, under the harsh conditions of mega-environments 1 (ME1: Cauquenes and Portezuelo). The ability of ‘Kauke’ to produce greater FY in low-fertility granitic soils is likely a result of its deep root system, which allows it to exploit soil water and nutrients at depths unreachable by annual crops and control cultivars. In a mesocosm study, Inostroza et al. (2023) demonstrated that alfalfa populations with putative drought tolerance exhibited different strategies of root biomass partitioning at 0-30 cm for dealing with drought stress. Some accessions decreased root biomass under water stress conditions, whereas the population CTA002 (‘Kauke’) did not change its root biomass across water treatments, indicating that this population possesses an intrinsically more robust root system.

The outstanding agronomical performance of ‘Kauke’ is fundamentally tied to its genetic background as an interspecific hybrid between *Medicago sativa* and its wild relative *M. arborea* (Bingham et al., 2013; Irwin and Bingham, 2023). *Medicago arborea* is a long-lived leguminous shrub adapted to the Mediterranean basin; it is known for its exceptional drought tolerance and ability to produce forage during both summer and winter. The introgression of *M. arborea* genes into the alfalfa genome has introduced several adaptive traits. For instance, a study by Tani et al. (2019) showed that, at the seedling stage, hybrid plants exhibited higher stem elongation rate, photosynthetic rate, stomatal conductance, and transpiration rate than the parental *M. sativa* and *M. arborea* accessions.

The annual forage distribution of ‘Kauke’ was found to be consistent with control cultivars, though the absolute quantities remained higher in the most stressed environments. In ME1, the concentration of 80% FY in spring is a classic drought-avoidance strategy, where the plant completes the majority of its vegetative growth before soil water potentials become critically low (Inostroza et al., 2015). The 20% FY obtained in January is particularly valuable, as it provides high-quality hay or grazing during the peak of the dry season. In contrast, the irrigated conditions in Chillán (ME2) allowed for a more balanced distribution, with 50% of the production occurring in the summer, highlighting the plasticity of the variety when water is not a limiting factor.

Fall dormancy (FD) is a critical determinant of alfalfa adaptation and productivity (Noushahi and del Pozo, 2026); is a physiological response triggered by shortening photoperiods and declining temperatures, resulting in reduced fall regrowth (Liu et al., 2019). Alfalfa cultivars are classified into 11 FD categories, broadly organized into three groups: (i) Fall-dormant types (FDT) (FD 1 to 4), (ii) semi-dormant types (SDT) (FD 5 to 7), and (iii) non-dormant types (NDT) (FD 8 to 11). The FDT cultivars exhibit early dormancy and reduced fall growth but possess high winter hardiness, ensuring persistence in higher-latitude environments. Conversely, NDT cultivars continue growing late into the fall; although they show lower winter survival, they achieve higher total annual yield due to their extended growing season. ‘Kauke’ is categorized as an SDT cultivar, which enables the plant to maintain growth into late autumn and resume early in the spring, increasing FY across the season.

Plant persistence is defined as the ability to maintain a viable plant population over several years (Ortega et al., 2018; Inostroza et al., 2020). Alfalfa stands naturally decrease over time, but the rate of decline is accelerated by drought, overgrazing, and soil nutrient deficiencies (Annicchiarico et al., 2015). The superior persistence of ‘Kauke’ in Cauquenes and Portezuelo, the sites with the longest dry periods, is a definitive indicator of its drought tolerance. In Cauquenes, the variety maintained a population density 40% higher than control cultivars by the end of the third growing season. This survival advantage is likely due to the ‘Alborea101’ influence, which provides a more robust crown and a deep, branched root system capable of surviving a 5 to 6 mo period without rainfall (Irwin and Bingham, 2023). Interestingly, ‘Kauke’ also showed a 40% density advantage in Vilcún, despite it being a high-rainfall site. This suggests that the variety's resilience extends beyond drought to include tolerance for anaerobic conditions or low temperatures associated with the cold and rainy springs of the Southern Mediterranean zone. This high survival rate underscores alfalfa's potential to provide long-term stability to forage production systems previously dominated by annual species.

CONCLUSIONS

Results from multi-location trials provided robust evidence that the new alfalfa 'Kauke' exhibits superiority in Mediterranean rainfed environments. Its 40% forage yield advantage in the most stressed environments (Cauquenes and Portezuelo), combined with its 40% higher plant density after 3 yr, establishes it as a cornerstone for future forage systems in the Mediterranean drylands of Chile. The semi-dormant growth habit (fall dormancy value 7) of 'Kauke' provides the necessary plasticity to capitalize on mild winter conditions while maintaining enough reserve stability to survive occasional thermal stress.

Author contribution

Conceptualization: L.I., C.O., A. del P. Methodology: L.I., C.O., A. del P., S.E., P.E. Formal analysis: L.I., G.M. Investigation: L.I., C.O., A. del P., S.E., P.E., V.B., G.M. Resources: L.I., C.O., A. del P. Data curation: L.I., G.M. Writing-original draft: L.I. Writing-review & editing: L.I., C.O., A. del P., S.E., P.E. Visualization: L.I. Supervision: L.I. Project administration: L.I. Funding acquisition: L.I., C.O., A. del P. All co-authors reviewed the final version and approved the manuscript before submission.

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