

UTILIZING GENESYS TO IDENTIFY WILD RELATIVES OF ALFALFA WITH ADAPTATION TO DIVERSE CLIMATES

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ABSTRACT

From its centers of origin, alfalfa (*Medicago sativa* L.) and wild relative populations have evolved to survive in highly diverse environments, with extremes that include the Arctic Circle, the desert areas of Kazakhstan and Western China, and the arid, Mediterranean areas of South Europe and North Africa. Alfalfa has further been disseminated anthropogenically, initially by ancient armies as a fodder source for horses, and in later European conquests to support emerging agriculture.

Here we demonstrate the use of Genesys, an online platform for housing information on global plant genetic resources, to identify a subset of alfalfa accessions collected from environments with bioclimatic variables linked to extreme drought, heat, and cold tolerance. The subset includes 28 alfalfa accessions originating from environments with an average monthly temperature range of -44–46 °C, 0–3,414 m elevation, up to 68.25 °N latitude and as low as 153 mm average annual precipitation (checked with satellite imagery to confirm no obvious supplementary water). The *M. sativa* subsp. represented in the subset include 14 subsp. *sativa*, 4 nothosubsp. *varia*, 1 subsp. *caerulea* and 9 subsp. *falcata*. The subset also contains 2 *M. sativa* subsp. *falcata* accessions collected from the extreme mildest winter temperature for this sub species, where the minimum temperature of the coldest month was at least 3 °C. The alfalfa climate adaptation subset, which is available for request from <https://www.genesys-pgr.org/subsets/0367d084-95c8-4d26-85d1-c14b98ebbb7b>, will now be characterised for key phenotypic traits and molecular diversity. The alfalfa and wild relatives assembled in this subset provide important unique diversity for a range of abiotic traits that can be introgressed into alfalfa to support carbon neutral farming and extend or maintain the range of alfalfa production for environments with changing climates.

GENESYS

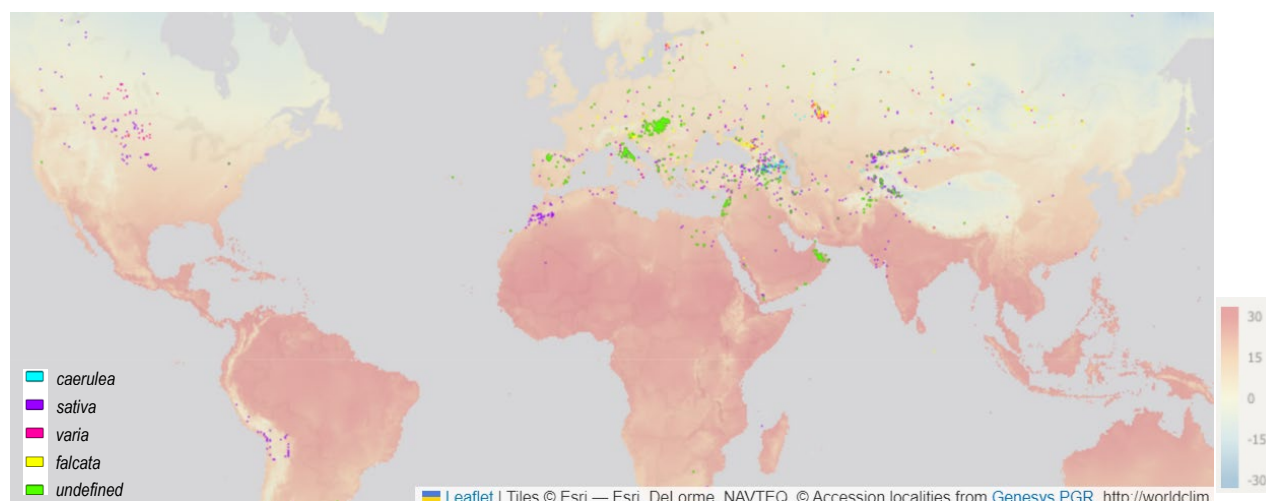
Genesys, <https://www.genesys-pgr.org>, is an online platform for managing information on global plant genetic resources, assembling databases from individual genebanks at one location. The mapping feature of Genesys displays the global distribution of collection origins for accessions that are georeferenced, proving excellent information on the known geographical distribution of a species (Figure 1). This feature allows the user to refine their search for accessions based on latitude, longitude, and elevation. The georeferenced data is also overlaid onto www.worldclim.org datasets, which further allows accession lists to be refined using a range of bioclimatic variables (rainfall, temperature, seasonality etc.).

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We used Genesys to summarize the geographical and climatic adaptation of *M. sativa* based on collection origins, and identify individual accessions collected from the most extreme environments. The accessions are available as a subset, hereafter ‘alfalfa diverse climate adaptation subset’ and can be viewed and requested as a group through <https://www.genesys-pgr.org/>.

ALFALFA ACCESSION DIVERSITY BASE ON COLLECTION ORIGIN

There are 19,736 alfalfa accessions held in international genebanks, with 2,494 of these accessions georeferenced (i.e., have latitude and longitude information, Table 1, Figure 1). The United States Department of Agriculture, National Plant Germplasm System (USDA NPGS) houses the largest collection of alfalfa germplasm (Irish and Greene 2021), followed by the Vavilov Institute of Research (VIR) and Australian Pastures Genebank (APG). Some duplication exists between the three genebanks (because of germplasm exchanges), with for example, 638 of the APG accessions being known duplicates with Plant Introduction (PI) or Western Regional Plant Introduction Station (W6) numbers. The introduction of Digital Object Identifiers (DOI) numbers, being considered by several national genebanks, will identify further duplication.



<i>M. sativa</i> subspecies*	NPGS	VIR ⁺	APG	Other	Total
<i>caerulea</i>	97 (41)	67 (0)	182 (122)	86 (9)	432 (172)
<i>sativa</i>	3,372 (920)	2,470 (0)	1,165 (161)	879 (49)	7,886 (1,130)
<i>varia</i>	437 (202)	714 (0)	412 (223)	676 (53)	2,239 (478)
<i>falcata</i>	457 (297)	335 (0)	303 (205)	301 (108)	1,396 (610)
<i>glomerata, tunetana, viscosa</i>	33 (13)	53 (0)	57 (27)	37 (0)	180 (40)
undefined	8 (0)	0 (0)	576 (32)	7,019 (32)	7,603 (64)
Total	4,404 (1,473)	3,639 (0)	2,695 (770)	8,998 (251)	19,736 (2,494)

*Current Germplasm Resources Information Network (GRIN) taxonomy. ⁺Classified as species not subspecies: *M. caerulea*, *M. sativa*, *M. falcata*. VIR accessions are not referenced on Genesys. *M. sativa* was used as *M. sativa* subsp. *sativa* for RUS001, consequently no accessions remain undefined. Information collated from Genesys-PGR and GRIN-Global databases. Background displays annual mean temperature.

Figure 1. The number and distribution of *Medicago sativa* L. subsp. accessions held at the NPGS, APG and other international genebanks, listed on Genesys (November 2022). Numbers in parenthesis indicate number of georeferenced accessions.

The adaptation of the *M. sativa* complex to a range of geographic and bioclimatic variables, based on the collection origin of georeferenced accessions, is shown in Figure 2. The subsp. *sativa* has the greatest range of adaptation, but these results are also skewed by the higher representation in genebanks.

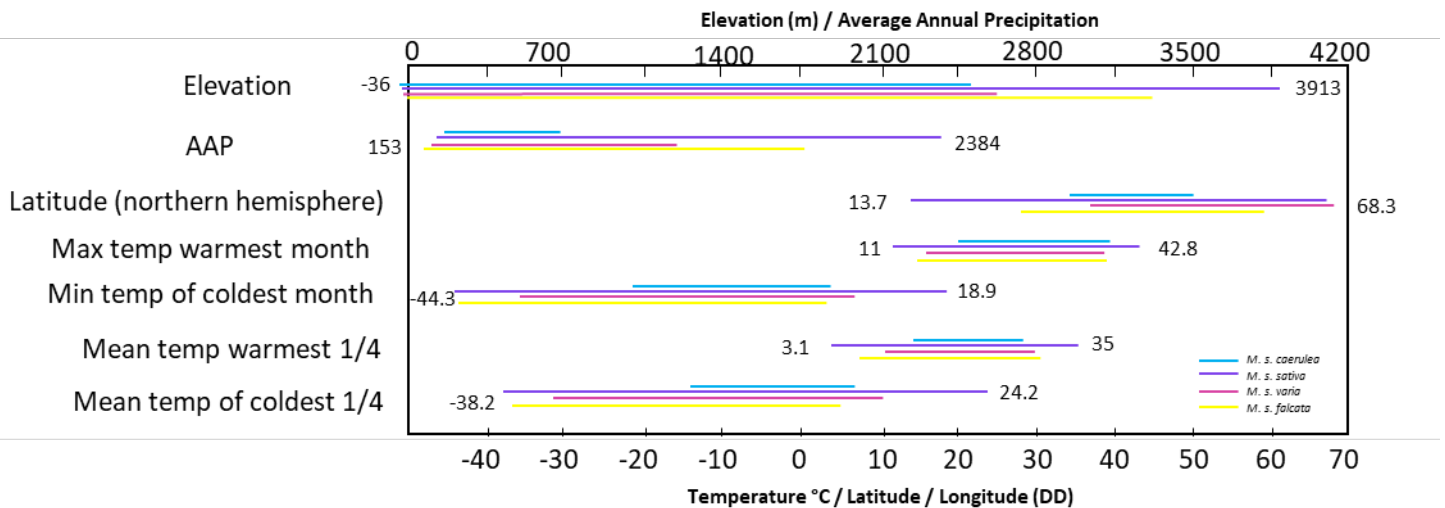


Figure 2. Adaptation of *Medicago sativa* L. subsp. accessions [wild, (natural, semi natural), traditional landrace and unclassified] to different environments based on elevation, average annual precipitation (AAP), northern hemisphere latitude, and bioclimatic variables that include the maximum temperature of the warmest month, minimum temperature of the coldest month, mean temperature of warmest quarter, and mean temperature of the coldest quarter. Extreme values at species level shown for each variable.

ALFALFA DIVERSE CLIMATE ADAPTATION SUBSET

The alfalfa diverse climate adaptation subset contains 28 accessions including 14 subsp. *sativa*, 4 nothosubsp. *varia*, 1 subsp. *caerulea* and 9 subsp. *falcata*. (Table 1). The subset has been developed to allow users to easily identify and request seed of accessions that represent extremes in adaptation of the species.

The success of the subset in achieving the goal of identifying accessions with extreme adaptation relies on the accuracy of the recorded georeference, which is known to have a degree of uncertainty. This uncertainty is particularly relevant when using this method to identify drought tolerance, because rainfall is inherently variable over short distances. We used Google Maps satellite imagery to identify accessions with low average annual precipitation that weren't obviously receiving supplementary water from irrigation or waterways, and this relies on a relatively accurate georeference. For this reason, we identify several accessions to represent extreme variability for each geographic and bioclimatic trait.

The APG is regenerating seed of the subset, and there are plans to have the whole subset available for distribution by June 2023 from both the APG and NPGS.

In addition, the climate adaptation subset will be evaluated for phenotypic and agronomic traits as well as molecular diversity. Data and results from all evaluations of this subset will be available at Germinate 3, <https://ics.hutton.ac.uk/cwr/alfalfa/#/home> and from GRIN-

Global. It is hoped that the climate adaptation subset is used together the original alfalfa core collection (Basigalup 1995) and any future core collections developed using modern genomic sequencing methods.

Table 1. Alfalfa (*Medicago sativa*) diverse climate adaptation subset.

Selection Criteria	OS	APG	NUMB	SUB-SP	CTY	LAT	LONG	ELV	AAP	ATR	TWM	TW 1/4	TCM	TC 1/4
1. Northern Latitude (LAT)	3,4,8	85092	PI 631833	falcata	SWE	68.25	13.83		1,341	18.3	13.9	10	-4	-2
		85104	ABY-Af 1430	falcata	RUS	65.30	115.94		303	65	21	12	-44	37
		85095	PI 251692	sativa	RUS	67.62	33.65		571	34	17	10	-17	-12
		85097	PI 452469	sativa	CAN	61.17	-113.67		302	51	21	14	-30	-23
2. Max Elevation (ELEV)		43047	W6 14166	varia	IND	34.14	77.56	3257	119	37	18	10	-20	13
		85088	PI 631612	falcata	NPL	28.82	83.85	3414	402	25	15	9	-10	-3
		85101	PI 632066	sativa	PAK	35.29	75.65	3277	139	39	16	9	-23	-17
		85102	W6 23584	sativa	CHN	31.33	100.73	3060	643	34	21	14	-13	-2
3. Ave Annual Precipitation (AAP)		16453	MJM 7318	sativa	IRN	35.87	51.47	1935	182	42	30	20	-12	-5
		21565	CPI 103195	varia	LBY	31.40	15.63	40	157	26	33	27	7	13
		38309	IFMI 2362	sativa	SYR	33.53	36.35		190	34	36	24	2	8
		43145	PI 384890	sativa	IRN	36.42	55.02	1700	163	35	33	25	-3	4
		84274	VIR 50713	varia	KAZ	47.84	59.62	173	183	51	32	23	-18	-12
		84837		caerulea	KAZ	47.72	56.06	85	195	49	33	24	-16	-10
		84976	PK-CWR-0035	varia	PAK	35.77	75.39	2534	162	39	26	18	14	-8
		35189	PI 499663	falcata	CHN	44.09	88.51		197	50	30	22	-20	-13
4. Annual Temp range (ATR)	1,8	85103	W6 40005	sativa	RUS	61.92	129.66		249	70	26	16	-44	-38
5. Max Temp warmest month (TWM)	6	38231	IG 101387	sativa	IRQ	36.35	43.12		466	41	43	31	2	9
		6742	PI 202824	sativa	SAU	24.23	47.37		87	33	43	34	10	17
		85094	PI 145202	sativa	SAU	21.43	39.82		69	25	42	35	17	24
6. Mean Temp warmest ¼ (TW1/4)		38322	IFMI 2427	sativa	OMN	23.67	57.83	0	75	24	40	34	16	21
		85096	PI 380916	sativa	IRN	27.27	53.60		156	28	37	31	9	16
		85100	PI 516841	sativa	MAR	29.82	-5.72		39	41	46	35	5	14
		85093	W6 39982	falcata	RUS	22.65	39.76		72	27	38	31	11	19
7. mild climate falcata		36133		falcata	GRC	39.16	23.49	56	497	28	32	26	4	9
	85085	PI 631584	falcata	ITA	45.65	13.78		1,077	24	28	23	3	6	
8. Min Temp coldest month (TCM)	3	85091	PI 631679	falcata	MNG	49.86	92.07		144	61	25	17	-36	-28
		85089	PI 631676	falcata	MNG	49.50	94.35		244	58	21	14	-37	-29

OS = other selection criteria accession matches in first column, APG = Australian Pastures Genebank number, Numb = other numbers, SUB-SP = *M. sativa* subspecies, CTY = country of origin, Lat = latitude, LONG = longitude, ELV = elevation, AAP = average annual precipitation, ATR = Temperature annual range (bio5-bio6) [°C], TWM = Max temperature of warmest month [°C], TW1/4 = Mean temperature of warmest quarter [°C], TCM = Min temperature of coldest month [°C], TC1/4 = Mean temperature of coldest quarter [°C]

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ACKNOWLEDGEMENT

This work was undertaken as part of the initiative “*Biodiversity for Opportunities, Livelihoods and Development*” (BOLD) which is supported by the Government of Norway. The project aims to strengthen food and nutrition security worldwide by supporting the conservation and use of crop diversity. BOLD is managed by the Global Crop Diversity Trust and implemented in partnership with national and international genebanks and plant breeding institutes around the world. For further information, go to the project website: <https://www.croptrust.org/work/projects/the-bold-project/>