Introgression of alfalfa crop wild relatives for climate change adaptation[*]

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ABSTRACT: Crop wild relatives (CWR) have evolved to survive in extreme environments, and have developed many different strategies for drought tolerance. This adaptation provides the potential to contribute novel alleles for the breeding of more resilient forages in the face of global climate change. Through our partnership with the Crop Trust, we aim to identify, conserve and introgress crop wild relatives of alfalfa into modern plant varieties. The new pre-bred lines have overcome issues with inter-specific hybridisation, allowing plant breeders to more easily introduce new diversity into their breeding programs. The ultimate aim is to breed new resilient varieties for developing countries that benefit subsistence and smallholder farmers, who are most vulnerable because they live in marginal food production environments and are least equipped to deal with change.

Extreme examples of drought tolerant alfalfa accessions were identified and acquired from the USDA-ARS (USA), Vavilov Institute (Russia), Kazakhstan and the Australian Pastures Genebank (APG). In addition, the most drought tolerant Russian ecotypes (or species using Russian taxonomy) described by Sinskaya (1950) were targeted to maximise diversity from this primary centre of evolution. Recent collection trips by Kew (UK), SARDI (Australia) and KSRIAPG (Kazakhstan) to Georgia, Italy, Azerbaijan and Kazakhstan have also provided access to new diversity.

This paper reports on progress with the development of new hybrid and pre-bred lines developed from crosses between alfalfa (*Medicago sativa* subsp. *sativa*) and *M. sativa* subsp. *falcata*, *M. sativa* subsp. *caerulea*, *M. arborea*, *M. strasseri*, *M. sativa* subsp. *glomerata* and *M. truncatula*, and highlights the free availability of all accessions and pre-bred lines for further research and development under the SMTA from the Australian Pastures Genebank.

1. Identification, acquisition and conservation of new CWR accessions with a focus on drought tolerance

The book 'Alfalfa and wild relatives' (Small 2011) was used to identify germplasm at a species level with the potential to contribute to drought tolerance and previous success at intra/inter species crosses with *M. s. sativa*. From this review, *M. prostrata, M. arborea, M. strasseri, M. s. falcata, M. s. caerulea* and *M. ruthenica* were identified as major sources of drought tolerance diversity.

Accessions from the APG and USDA genebanks with putative drought tolerance were selected based on their passport data, including average annual rainfall combined with location and collection notes. Accessions were also selected if other harsh environmental conditions such as salt tolerance (increasing osmotic stress), grazing tolerance or evolution with competitive rhizomatous grasses could be identified. KSRIAPG have also recently donated 62 accessions to the APG from arid environments in Western Kazakhstan (Table 1).

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As part of the CWR program, Kew has conducted targeted collection missions to conserve crop wild relatives in environments that are underrepresented in genebanks and most at risk of local extinction in the face of climate change. Recent plant collections in collaboration with the Universita degli Studi di Pavia (Italy) and the National Botanical Garden of Georgia (NBGG, Republic of Georgia) have resulted in the acquisition and conservation of new alfalfa genetic resources (Table 1).

The Russian 'Flora of cultivated plants of the USSR' (Sinskaya 1950) book was used to identify ecotypes (or species using the Russian taxonomy) with extreme sources of drought tolerance in *M. s. falcata* and *M. s. caerulea* (Table 2). Accessions representing these ecotypes were acquired by APG from the N. I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) in 2017, with seed now available under a SMTA (Table 2).

Table 2. *Medicago sativa* ecotypes specifically mentioned with high levels of drought tolerance in 'Flora of cultivated plants of the USSR' (Sinskaya 1950)

Ecotype description	sub species	APG Accession
South Russian Steppe Regional ecotype – Ukraine, central	*falcata*	84259, 84260, 84261, 84262
Caucasian foothill steppe ecotype	*falcata*	84270, 84271
South Russian floodland ecotype – Kubin	*falcata*	842645,84266
Siberian Steppe regional ecotype – variety Omskaya 2251	*falcata*	84267
Western Siberian forest steppe ecotype, Leningrad Oblast deep rooted	*falcata*	84268, 84269
***Medicago trautvetteri* eastern ecotype**	*varia*	84272, 84273
***Medicago trautvetteri* western ecotype**	*varia*	84274, 84275

Two additional ecotypes, 'Altyn Emel' and 'Ural root suckering' (from Western Siberia) were not sourced.

2. Introgression of CWR into *M. sativa* subsp. *sativa* plant varieties

Crop wild relatives of alfalfa targeted in this project are being introgressed into alfalfa varieties, with the aim of developing a cohort of pre-bred lines for use in alfalfa breeding programs around the world. A list of the new lines developed to date are shown in Table 3.

New lines developed from crosses between *M. s. caerulea* and alfalfa were made using both diploid (relying on the production of 2n pollen) and tetraploid versions of accession APG 42382. This accession was targeted because it was collected from the most arid site in Azerbaijan (the Abseron peninsula) where this species was represented (Auricht et al. 2009). Drought tolerance may be associated with ploidy in this diploid subspecies of sativa, representing a challenge for plant breeders working in tetraploid *M. s. sativa*.

A total of 55 new lines have been made between *M. s. falcata* and *M. s. varia* (*M. trautvetteri*) and alfalfa varieties from Australia, China and Kazakhstan. The accessions in these crosses were obtained from VIR, identified by N. Dzyubenko (Pers. Comm 2017), representing drought tolerant ecotypes (in Table 1) described by Sinskaya (1950). A pre-bred line developed earlier (CTA011, Table 3) has shown promise for high yield production at Almaty in Kazakhstan, indicating the potential success of introgressing CWR into modern varieties. This CWR accession in this cross co-existed in a dense sward with *Agropyron.* We are interested in the potential of the *M. s. falcata* subspecies to improve the compatibility of alfalfa in mixtures with perennial grasses, and thus extend the production of alfalfa into permanent pastures.

The development of *M. sativa* x *M. arborea* hybrids (called Alborea) has been pioneered by Edwin Bingham (2009). The potential of Alborea in restructuring a number of traits in alfalfa has also been reported by Irwin et al. (2016). Bingham has kindly donated the population Alborea-101, which we started to seed increase with a 500 plant population, employing a single seed descent method over five generations to promote gene mixing and the development of new phenotypes following tetrasomic inheritance. Seed of earlier generations will also be available from the APG under the SMTA. In addition, we have also attempted our own crosses between 22 accessions of *M. arborea* plants (15 year old plants growing at the Waite Institute) and alfalfa in 2017/18. We did not use a male sterile mid-parent as described by Bingham (2009), hoping to maximise low fall dormancy (maximum winter or fall growth rates). Our crosses concentrated on using high seed yielding, non-fall dormant alfalfa (class 10) germplasm from Australia and the USA.

Table 1. Summary of accessions identified and acquired with new diversity for drought tolerance**.**

Taxon	No. accessions	Key APG* accessions	Country / region	Donators	Collection / breeding notes of key accessions
M. arborea	24	-	Greece	1	15 year old plants at the Waite Institute
M. ruthenica	1	43029	China, Inner Mongolia	2	Selected for erect habit
M. sativa subsp. *sativa*	1	Patagonian alfalfa	Chile	10	Seed collected from 80 year old plants growing in Patagonia Chile
	1	16453	Iran	1	Low rainfall, shallow soil (MJM 7318)
	1	6567	Spain	1	YI-1, selection made at Aula Del, Zaragoza for arid zones
M. s. caerulea	1	42382	Azerbaijan, Nardaran	1	Very dry, hot and overgrazed by sheep.
	10	84831, 84834	West Kazakhstan, Aktobe	3	Arid sand to sandy loam sites
M. s. falcata	17	38808, 38116, 38690	Kazakhstan Ayagoz	1, 4	Co-existence with *Agropyron* spp., saline, soil pH 10
	9	854, 856	Italy	5,6	High relative yield under rainfed / irrigation.
	19	84767, 84775	West Kazakhstan, Aktobe	7, 3	Mugalzhar mountains, Khromtau district, in association with *Agropyron* and *Astragalus*.
M. s. glomerata	*10*	84245, 84247, 84248	Georgia	8	CWR 867308. Good biomass, large flowers
M. s. varia	43	84779, 84781, 84805	West Kazakhstan, Aktobe	7, 3	Feathergrass (*Stipa*) and *Agropyron*.
M. strasseri	1	41909	Spain (ex Greece)	9	Improved leaf holding capacity relative to M.arborea.

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Table 3. New pre-bred lines and hybrids developed

APG or project identifier*	Taxon	Breeder	Fall dormancy	Parents / Description
APG422, APG4293, APG4294	*M. sativa* subsp. *varia*	Humphries	2-4	Field selections for persistence in South Australia. Diverse background
CTA007-CTA011	*M. sativa* subsp. *varia*	Humphries	2-4	Five lines FD7 lucerne x Kazakhstan (38690, 38815-18 *M. s. falcata* from saline areas with *Agropyron*) backcrosses back into *M. s. sativa*
CTA013	*M. sativa* subsp. *varia*	Humphries	2-4	32 lines, FD7 lucerne (broad) x VIR falcata APG84261, 84269, 84270 with high yield
CTA014	*M. sativa* subsp. *varia*	Humphries	2-4	33 lines, FD7 lucerne (broad) x VIR "trautvetteri" APG84272, 84273, 84274, 84275 with high yield
CTA015	*M. sativa* x *M. s. glomerata*	Humphries	2-4	22 lines, FD7 lucerne (broad) x *M. s. glutinosa* APG84245, 84247, 84248 (large flowers, moderate yield)
CTA016	*M. truncatula* 4x	Humphries	n/a	28 tetraploid seed lines doubled from *M.truncatula* cv. Sultan SU
CTA017	*M. sativa* x *M. truncatula*	David Peck	?	Tetraploid Tet1-2 *M. truncatula* x SARDI 7Series 2 lucerne. Three plants.
Alborea-101	*M. sativa* x *M. arborea*	Edwin Bingham	3-10	Alborea composite line. 500 plant population
CTA019	*M. sativa* x *M. arborea*	Edwin Bingham	9-10	Alborea crosses, FD 9-10. W10 x Genesis
CTA020	*M. sativa* x *M. arborea*	Alan Humphries	?	181 seed lines from 22 Arborea accessions x FD7 and FD10 lucerne varieties crossed in 2017
CTA021	*M. sativa* x *M. strasseri*	Alan Humphries	?	66 seed lines from APG41909 x FD7 and FD10 lucerne varieties

*All lines will be given an APG number once seed is available for deposit, APG = Australian Pastures Genebank.

This decision resulted from the impressive growth rates of a M10 x Genesis hybrid (Genesis is a FD7 cultivar), also provided to us by Bingham. The F1 generation will be screened for habit, height, stem thickness and flower colour (yellow flowers are inherited from *M. arborea*) to confirm that true hybrids have been made.

In the same approach we have also attempted to make crosses between alfalfa and *M. strasseri* accession APG41909. *M. strasseri* is closely related to *M. arborea*, and is only found on the island of Crete (Greece). This is likely to be the first time this cross has been attempted, given rareness of this species, with only three accessions housed in genebanks around the world (Small 2011).

Hybrids between alfalfa, *M. truncatula* and *M. intertexta* have also been reported to be successful (Bingham Pers. Comm. 2017). We plan to evaluate and multiply a mixed population of *M. sativa* x *M. truncatula* x *M. intertexta* (referred to as Perennial X Annual Crosses or M.PAC) from Bingham. *M. intertexta* is a high yielding annual species that could be of interest to Australian farmers in low rainfall Mediterranean environments, but is not grown because of large spines on its pods. We are also confident that we have three *M. sativa* x *M. truncatula* (tetraploid following chromosome doubling) hybrid plants. The plants have yellow flowers and an appearance that is close to the tetraploid *M. truncatula* parent (albeit with larger flowers), and look to have impressive forage yield. One of the three putative hybrids has stable pollen. The three plants (and vegetative clones produced from them) will be crossed back into non-dormant alfalfa and evaluated for perenniality in 2018/19.

Evaluation and free distribution of new hybrid lines for plant breeders. The individual APG accessions and hybrid lines described in this paper will be made freely available to researchers through the APG under an SMTA as consistent with the International Treaty on Plant Genetic Resources for Food and Agriculture. A cohort containing the main outputs of the project will be available under the name 'CT_alfalfa cohort' from June 2020. Seed can be ordered online from the APG public web site at https://apg.pir.sa.gov.au/gringlobal. The cohort is being evaluated by the project partners in South Australia, central and southern Chile, south and north Kazakhstan and in Inner Mongolia, China (some early results presented in posters at this conference). Full details of pre-bred lines and associated data can soon be found on Germinate 3 at **https://ics.hutton.ac.uk/cwr/alfalfa/#home**.

The aim of the Crop Trust CWR alfalfa project is to promote the availability of these new hybrids and pre-bred lines in breeding programs around the world, with the understanding that will take a collaborative effort to develop new resilient varieties adapted to a changing climate.

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