

Wagner, M. H., Aïssiou, F., Badeck, F. W., Deniot, G., Ducournau, S., Dupont, A., ... & Chèvre, A. M. (2022, August). Genetic diversity in Mediterranean Brassica vegetables: seed phenotyping could be useful for sustainable crop production. In *XXXI International Horticultural Congress (IHC2022): International Symposium on Conservation and Sustainable Use of 1384* (pp. 173-182).

The original publication is available at: [10.17660/ActaHortic.2023.1384.23](https://doi.org/10.17660/ActaHortic.2023.1384.23)

IHC2022 - S2 CONSERVATION AND SUSTAINABLE USE OF HORTICULTURAL GENETIC RESOURCES

Genetic diversity in Mediterranean Brassica vegetables: seed phenotyping could be useful for sustainable crop production

Wagner MH¹, Aïssiou F⁸, Badeck FW⁴, Deniot G.², Ducournau S¹, Dupont A¹, Falentin C², Gay L³, Geraci A⁷, Glory P.², Hadj-Arab H⁸, Jarillo José A⁵, Meglic V⁶, Morcia C⁴, Pipan B⁶, Sinkovic L⁶, Oddo E⁷, Pernas M⁵, Pineiro M⁵, Richer V², Schicchi R⁹, Terzi V⁴, Chèvre AM²

¹GEVES, Station Nationale d'Essais de Semences, F-49071 Beaucozú cedex, France

²IGEPP INRAE, Institut Agro, Université de Rennes, F-35650 Le Rheu, France

³AGAP, INRAE, Arcade, 10 rue Arthur Young, F-34000 Montpellier, France

⁴CREA-GB, Council for Agricultural Research and Economics, Research centre for Genomics & Bioinformatics, via S. Protaso, 302, I-29017 Fiorenzuola d'Arda PC, Italy

⁵Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM)–Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC), Campus Montegancedo UPM, 28223 Pozuelo de Alarcón (Madrid), Spain

⁶Agricultural Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana, Slovenia

⁷Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, via Archirafi 38, I-90123, Palermo, Italy

⁸University of Sciences and Technology Houari Boumediene USTHB, Faculty of Biological Sciences FSB, Laboratory of Biology and Physiology of Organisms LBPO, Bab-ezzouar, BP 32 El-Alia, 16111 Algiers, Algeria.

⁹Department of Agricultural, Food and Forest Sciences, University of Palermo, viale delle Scienze Ed. 4, I-90128, Palermo, Italy

Abstract

A European funded project (H2020 PRIMA program) aims to explore the genetic diversity present in two economically important Brassica crop species, *Brassica oleracea* and *B. rapa*, for sustainable crop production. This diversity is present in wild populations but also in cultivated landraces and has been shaped by contrasting environments. An international consortium of 11 partners has begun to collect and multiply wild populations extending from the French North Atlantic coast to the southern Algerian desert as well as local varieties from 6 contributing countries in order to characterize the genetic diversity available over a wide soil-climate gradient.

A total of one hundred populations has been obtained for each species. Identifying the genetic variation and understanding the basis for it will allow the development of breeding strategies for a better adaptation of turnip (*B. rapa*) and cabbage (*B. oleracea*) to climate change. One third of the collection has been already phenotyped for its germination traits of native seeds harvested in 2020 for wild populations or of local landraces provided by farmers and seed banks. In favorable conditions, a high diversity in germination capacity and germination rate was observed independently of seed age. The two species have a different germination profile: some turnip seeds can stand higher temperature and lower water potential than most cabbage seeds. Variation in flowering time has also been documented in these plants. Furthermore, a second set of seeds of the full collection produced by IGEPP under common

environmental conditions will be phenotyped under three abiotic conditions for sowing using automated germination tools: one control condition, one single stress with high temperature and one double stress combining high temperature and water stress to simulate the effect of climate change in the Mediterranean from where the two species are native.

Keywords: cabbage, turnip, germination, wild populations, landraces, accessions, biodiversity, abiotic stress

INTRODUCTION

BrasExplor project: Wide exploration of genetic biodiversity for sustainable crop production

The BrasExplor project has begun in September 2020 joining 11 partners; it aims to collect, explore and characterize the genetic diversity of wild and cultivated forms of Brassica vegetable crops under different climatic constraints in order to develop genetic resources for these species as well as conservation strategies, to promote the use of local landraces and to develop new well-adapted varieties. This diversity of wild forms and landraces locally selected has been poorly explored until now (Aissiou et al., 2018). The two species concerned by the project are cabbage (*Brassica oleracea*, CC, 2n=18) and turnip (*B. rapa*, AA, 2n=20). Both species are native of the Mediterranean basin (Qi et al. 2017; Bird et al. 2017) and are important vegetables all over the world (Cheng et al., 2016).

The physiological response to high temperature and drought will be investigated in wild and locally cultivated populations focusing on traits ranging from seed germination and seedling establishment to flowering time and production. Early developmental stages can be phenotyped with a higher throughput (Demilly et al., 2014, Hatzig et al., 2018) and their responses to abiotic stresses have been used to screen tolerant lines for breeding (Finch-Savage et al., 2010; Beacham et al., 2016). They usually match with stresses common at the geographical origin of the species (de Jong et al., 2013; Gardarin et al., 2017), where especially temperature modulates between-species differences. Due to current anthropogenic climate change plant breeding needs to take in account global warming and seasonal disruption to secure crop production. An inventory of genetic resources is required for genetic improvement.

The preliminary study presented here is focused on germination plasticity through a first panel of *Brassica oleracea* and *Brassica rapa* genotypes coming from seedbanks accessions for landraces and from collected plants for wild populations.

MATERIALS AND METHODS

An automated high throughput phenotyping platform (Demilly et al., 2014) was used to assay diverse traits, related to the three different phases of seed germination and post-germination radicle growth, in a first set of 32 *Brassica oleracea* (BO) and 28 *B. rapa* (BR) genotypes. Four commercial samples were first used to calibrate the automated tools and define a common favourable temperature for both Brassica species. Then 21 wild populations (7 BO and 14 BR) collected during 2020 from the North of France to the Saharan region of Algeria and 39 landraces (25 BO and 14 BR) provided by Genetic Resources Seedbanks or collected from France, Italy, Algeria and Slovenia were phenotyped under two temperature regimes.

Seeds were sown on filter paper and incubated in Copenhagen tables equipped with RGB cameras integrated in automated germination tools. Trials were carried out from 4 to 14 days according to temperature: a range of temperature from 15 to 35°C for the commercial varieties and at 25°C for the collected material. In total, 100 seeds per genotype were analysed in four replicates (25 seeds per replicate). From the time progress curves main seed traits as

mean germination time (MGT), time to reach 50% of germination (T50), uniformity and germination ability were extracted. Germination rate was calculated using T50 and statistics included the mean, standard deviation, range, minimum, and maximum values in addition to variance analysis using SAS 9.4.

RESULTS

The two species could germinate in the range 15-35°C but germination rate of commercial seed lots was highest between 20 and 25°C for *Brassica oleracea* (Fig.1a) while *Brassica rapa* germinated faster between 25 to 30°C (Fig. 1b). At 35°C, germination rate still increased for *B. rapa* but variability between and within varieties increased at high temperature (Fig. 1b).

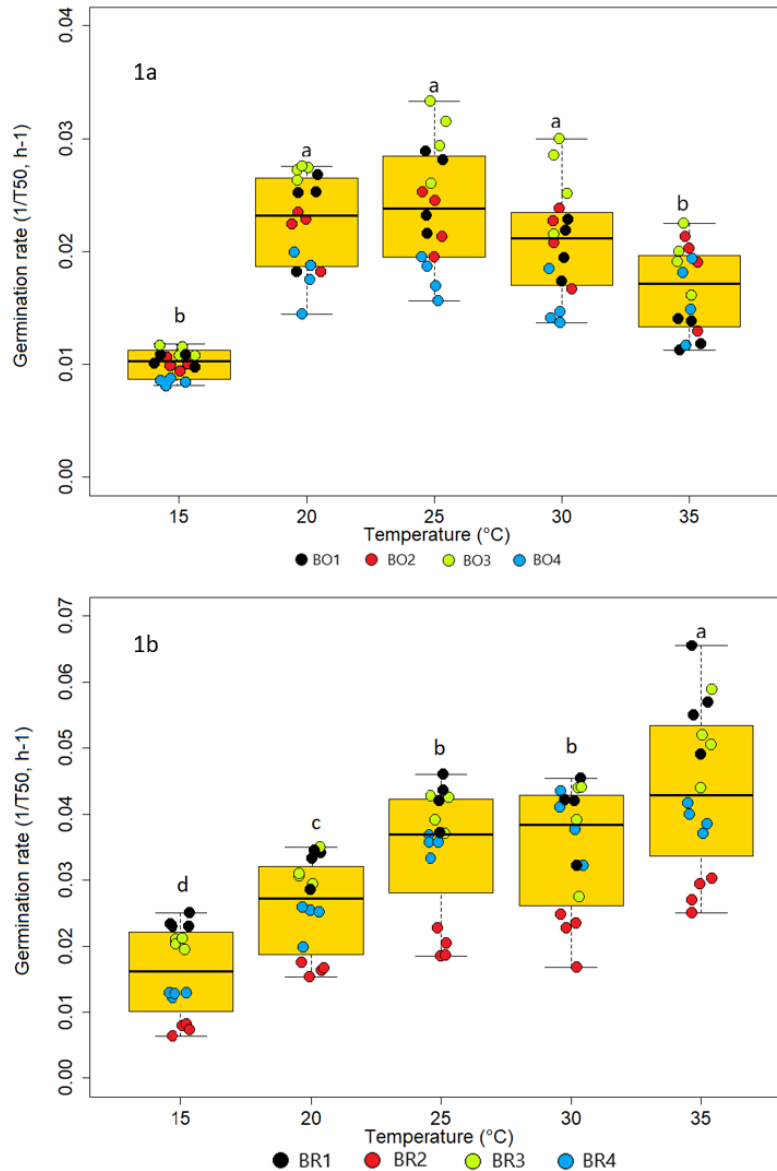


Figure 1: Germination response to temperature for 4 commercial seed lots of cabbage (1a) or turnip (1b). Box-plots with different letters are significantly different at $p < 0.05$ (Tukey HSD test)

For the first screening of genetic resources from BrasExplor project, germination has been tested at 25°C for the two species.

Germination ranged between 32 to 100% at 25°C. Only four accessions of *B. oleracea* germinated with less than 80% (Tab. 1), 3 of them were landraces and 1 wild population. Landraces and wild populations did not differ in germination rate (T50 around 40h) or capacity (90%).

Table 1: Germination main traits (mean germination time MGT, time to reach 50% of germination T50, uniformity or slope of germination time progress curve using T80 minus T20 and % of germination) for 32 *Brassica oleracea* sown at 25°C. Accessions are ranked from the lowest to the highest germination (mean of 4 x25 seeds). In white background, some landraces and in grey, the wild populations collected in 2020.

Country	Accession	Year	MGT (h)	T50 (h)	Uniformity T80-T20 (h)	Germination (%)
France	BO_F_OMER_L_A	2002	69,3	NaN	NaN	42,7
Slovenia	BO_S_LJUB_L_O	2016	45,6	97,0	NaN	54,7
Slovenia	BO_S_LJUB_L_L	2018	51,8	61,0	NaN	68,0
France	BO_F_TREP_W_A	2020	49,9	54,3	NaN	74,7
France	BO_F_BOVE_L_A	2006	53,2	55,0	48,5	81,3
Italy	BO_I_GALA_L_B	2018	55,2	54,3	57,3	82,7
France	BO_F_MARA_L_A	2000	48,0	45,8	35,0	86,7
Italy	BO_I_SOLE_L_C	2018	37,9	34,3	29,3	89,3
Slovenia	BO_S_LJUB_L_D	1991	34,4	28,8	30,4	89,3
Slovenia	BO_S_LJUB_L_G	2016	41,8	38,4	47,0	89,3
Slovenia	BO_S_LJUB_L_A	2010	41,0	37,0	36,7	92,0
Slovenia	BO_S_LJUB_L_C	1992	33,0	29,4	22,7	92,0
France	BO_F_PENL_W_A	2020	44,6	43,6	22,2	93,3
Slovenia	BO_S_LJUB_L_H	2013	29,8	26,3	17,4	93,3
France	BO_F_MORT_W_A	2020	46,6	41,8	29,0	94,7
France	BO_F_PLER_W_A	2020	32,8	30,2	18,0	94,7
France	BO_F_ANTI_W_A	2020	54,4	49,5	40,3	94,7
France	BO_F_JOUY_L_A	2013	31,6	28,6	15,4	96,0
France	BO_F_VAUC_W_A	2020	44,5	42,6	23,6	97,3
France	BO_F_GRAN_W_A	2020	35,2	31,5	24,5	97,3
France	BO_F_LOON_L_A	2008	38,0	36,1	12,0	97,3
Slovenia	BO_S_LJUB_L_J	2013	43,3	38,5	29,0	97,3
France	BO_F_PAIM_L_A	2011	49,5	45,5	25,7	98,7
France	BO_F_NEUV_L_A	2013	33,1	31,4	13,8	98,7
Algeria	BO_A_TAZL_L_A	2020	30,8	28,7	11,2	98,7
Slovenia	BO_S_LJUB_L_E	1991	38,2	33,2	20,8	98,7
France	BO_F_DOUA_L_B	2003	37,4	33,1	14,3	100,0
France	BO_F_SAEN_L_A	2015	28,6	25,4	14,5	100,0
Algeria	BO_A_BOUN_L_A	2020	27,3	24,2	15,0	100,0
Italy	BO_I_CORI_L_A	2018	29,5	25,1	16,5	100,0
Slovenia	BO_S_LJUB_L_I	2017	21,6	18,8	9,3	100,0
Slovenia	BO_S_LJUB_L_N	2018	40,5	35,0	29,4	100,0
Wild populations means		2020	44,0	41,9	26,3	92
Landraces means		2010	39,6	38,0	25,1	90

Regarding turnips, the second *Brassica* species, 15 accessions had a low germination most of which were wild populations collected in 2020 (Tab.2). No relationship was obtained between seed age and germination as the oldest accession collected in 1984 germinated at 93% and accessions with the lowest germination rate were collected in 2020. All wild populations were found in the first half ranking with a final germination below 90%. Their bulk mean was 62% while landraces of *B.rapa* germinated at 90%.

Table 2: Germination main traits (mean germination time MGT, time to reach 50% of germination T50, uniformity or slope of germination time progress curve using T80 minus T20 and % of germination) for 28 *Brassica rapa* sown at 25°C Accessions are ranked from the lowest to the highest germination (mean of 4 x25 seeds). In white background, the landraces and in grey, the wild populations collected in 2020.

Country	Accession	Year	MGT (h)	T50 (h)	Uniformity T80-T20 (h)	Germination (%)
Italy	BR_I_CAST_W_B	2020	55,0	NaN	NaN	32,0
Italy	BR_I_ALTO_W_A	2020	80,3	NaN	NaN	40,0
Algeria	BR_A_CHLE_W_A	2020	83,0	NaN	NaN	49,3
France	BR_F_FOUG_W_A	2020	38,5	97,0	NaN	50,7
Algeria	BR_A_TZRA_L_A	2015	80,6	111,7	NaN	56,0
Italy	BR_I_MISI_W_B	2020	46,6	52,4	NaN	56,0
Italy	BR_I_GRAT_W_B	2020	61,4	77,0	NaN	56,0
Algeria	BR_A_REGH_W_A	2020	58,3	67,7	NaN	56,0
Algeria	BR_A_TIBE_W_A	2020	68,4	73,6	NaN	57,3
Algeria	BR_A_SETI_L_A	2016	84,2	97,0	NaN	70,7
Algeria	BR_A_ORAN_W_A	2020	65,8	55,9	NaN	72,0
Algeria	BR_A_HAYO_L_A	2018	39,9	37,0	NaN	74,7
Algeria	BR_A_TLMC_W_A	2020	49,3	43,2	NaN	77,3
Italy	BR_I_MARI_W_A	2020	64,1	48,4	NaN	78,7
Algeria	BR_A_BARA_W_A	2020	48,8	39,8	NaN	78,7
France	BR_F_MORD_W_A	2020	27,9	28,5	31,7	81,3
France	BR_F_RENN_W_A	2020	29,4	25,8	23,0	86,7
Slovenia	BR_S_LJUB_L_B	2013	32,2	25,0	27,8	90,7
France	BR_F_AUGN_L_A	2006	23,4	20,7	10,3	92,0
France	BR_F_BOUS_L_A	1984	30,8	26,8	19,7	93,3
France	BR_F_COMB_L_A	2004	25,9	23,3	14,1	94,7
France	BR_F_PARC_L_A	2014	25,0	21,3	12,2	94,7
France	BR_F_LAUR_L_A	2004	33,2	26,5	23,7	96,0
France	BR_F_GREN_L_D	2015	21,4	18,2	7,8	98,7
France	BR_F_SAVI_L_A	2015	22,8	20,6	9,8	98,7
France	BR_F_PONT_L_B	2008	22,1	19,1	8,5	100,0
France	BR_F_PLOB_L_B	2015	20,6	18,2	8,6	100,0
France	BR_F_HERB_L_A	2007	23,1	21,1	7,8	100,0
Wild populations means		2020	55,5	55,4	27,3	62
Landraces means		2010	34,7	34,7	13,6	90

Germination spread was larger than previously monitored on commercial seed lots (red and black dotted curves) as shown on figures 2 for *B. oleracea* and 3 for *B. rapa*.

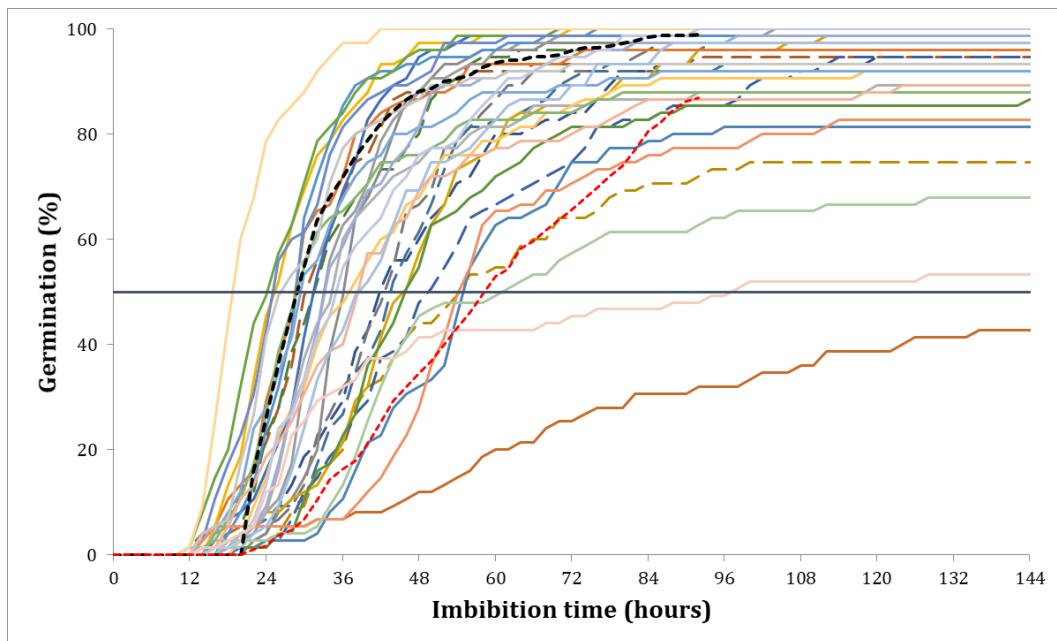


Figure 2: Automated germination time progress curves at 25°C for 32 *Brassica oleracea* with 25 landraces in full lines and 7 wild French populations with dotted lines.

Germination spread was larger for *B. rapa*. Interestingly, all wild populations of *Brassica rapa* collected in 2020 had the lowest and slowest germination (Fig.3).

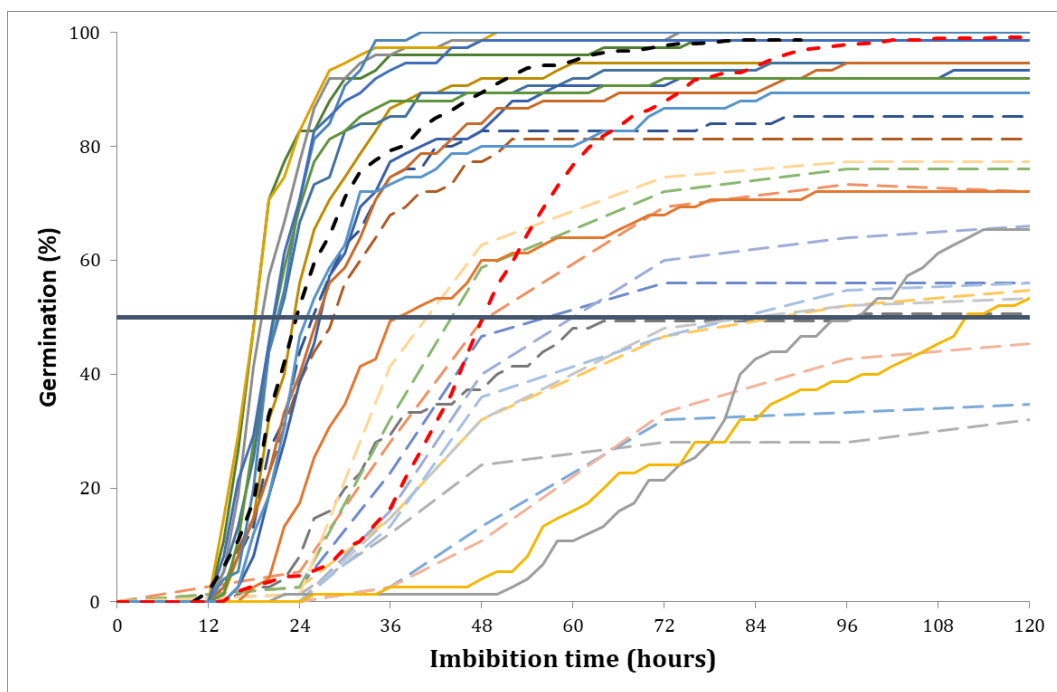


Figure 3: Automated germination time progress curves at 25°C for 28 *Brassica rapa* with 14 landraces in full lines and 14 wild populations with dotted lines.

These wild populations presented hard seed coats which needed to be scarified to release seed dormancy. They were mainly collected in dry regions from Algeria and Sicily with monthly precipitations below 40 mm (Tab.3). Drier seed maturation conditions correlated with lower germination and slower germination rates.

Among landraces which germinated above 80% after 5 days at 25°C, some could keep their high germinability at 35°C (Fig.4). As expected, *Brassica rapa* were more tolerant than *B. oleracea* but one of this later, BO_F_DOUA_L_B, reached 90% of germination at high temperature. A screening for warm adaptation could be set up based on this differential behaviour for temperature.

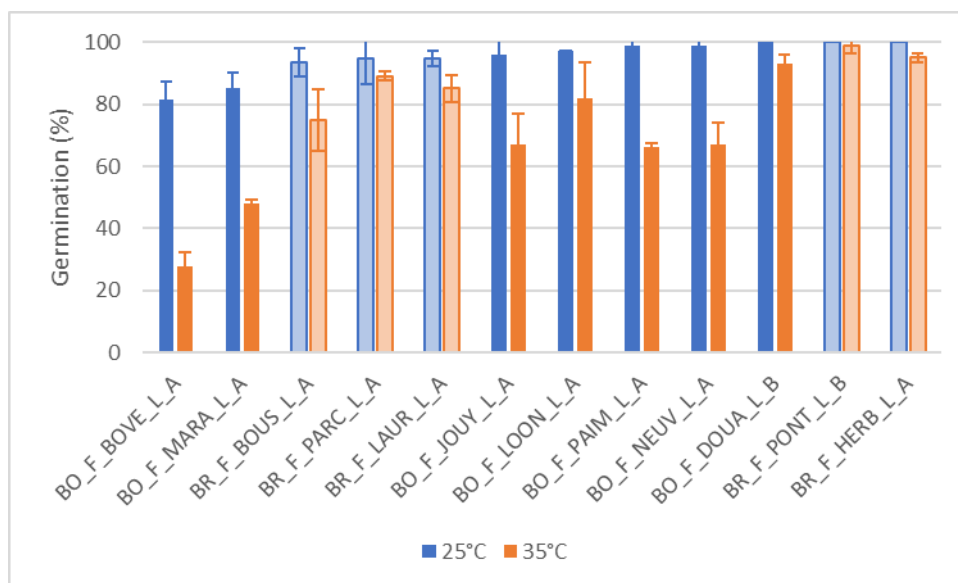


Figure 4 : Mean germination (\pm SD of 4 x 25) of 12 landraces provided by BraCySol GRC in France. 7 *B. oleracea* BO, dark coloured and 5 *B. rapa* BR, light coloured, at two temperatures: 25°C in blue bars and 35°C in orange bars.

Table 3: Worldclim database extraction during the period 2010-2018 for each location where local landraces or wild populations were collected during 2020.

Species	Accession name	Collection site	Mean monthly precipitation (mm)	Monthly mean temperature (°C)		Mean Germination Time (h)	Germination (%)
				Min T°	Max T°		
<i>Brassica oleracea</i>	BO_A_BOUN_L_A	Bouni (Algeria)	51,6	10,3	20,2	27	100
	BO_A_TAZL_L_A	Tazla (Algeria)	48,0	10,6	20,5	31	99
	BO_F_ANTI_W_A	Cap d'Antifer (France)	62,8	9,0	14,5	54	95
	BO_F_GRAN_W_A	Granville (France)	62,9	9,3	15,7	35	97
	BO_F_MORT_W_A	Mortagne (France)	67,3	9,2	18,3	47	95
	BO_F_PENL_W_A	Penly (France)	63,7	8,0	14,4	33	95
	BO_F_PLER_W_A	Plérin (France)	73,8	8,9	15,9	29	100
	BO_F_TREP_W_A	Le Tréport (France)	61,6	8,2	14,5	50	75
	BO_F_VAUC_W_A	Vaucottes (France)	64,2	8,6	14,6	45	97
<i>Brassica rapa</i>	BR_A_BARA_W_A	Baraki (Algeria)	59,3	13,1	24,3	49	79
	BR_A_CHLE_W_A	Chlef (Algeria)	35,7	13,3	25,6	83	52
	BR_A_ORAN_W_A	Oran (Algeria)	32,5	12,7	23,5	66	72
	BR_A_REGH_W_A	Réghaïa (Algeria)	58,0	12,7	23,6	58	56
	BR_A_TIBE_W_A	Tiberkanine (Algeria)	43,5	12,8	25,1	68	57
	BR_A_TLMC_W_A	Tlemcen (Algeria)	47,3	8,7	21,8	49	77
	BR_F_FOUG_W_A	Fougeré (France)	56,6	7,9	17,1	39	51
	BR_F_MORD_W_A	Mordelles (France)	63,1	8,3	16,4	28	81
	BR_F_RENN_W_A	Rennes (France)	61,3	8,1	16,7	29	87
	BR_I_ALTO_W_A	Altofonte (Italy)	41,6	11,8	18,3	80	40
	BR_I_CAST_W_B	Castelbuono (Italy)	37,0	13,4	20,6	55	32
	BR_I_GRAT_W_B	Gratteri (Italy)	39,3	12,6	19,4	61	56
	BR_I_MARI_W_A	Marineo (Italy)	37,0	14,0	21,1	64	79
	BR_I_MISI_W_B	Misilmeri (Italy)	36,3	14,5	21,6	47	56
Overall mean			52	10,7	19,3	49	75
Range			32-74	8-14	14-26	27-83	32-100

In order to characterize only genetic variation, all the material collected in 2020, the first year of the project, has been produced in the same place (INRAE) for a second round of phenotyping from germination (GEVES) to flowering (INIA) and cold acclimation (CREA). Based on the climatic data collected during the last decades (Tab.3) and in order to impose common conditions between experiments, the reference conditions for germination were chosen to be 20°C for both *Brassica oleracea* and *Brassica rapa*.

DISCUSSION

First results show that there is diversity in germination traits and data are consistent with previous studies (Castillo et al., 2019). Plasticity of germination traits exists but should be checked with seed batches produced in the same conditions.

Hardseededness has not been often described in *Brassica* (Baskin and Baskin, 1998) and this seed coat-imposed dormancy should be investigated in relation to seed origin (dry areas).

Both wild and domesticated biodiversity in the Mediterranean agricultural area have been studied in BrasExplor and accessions grow originally in highly contrasted habitats (altitude, temperature, pluviometry, soil structure and diversity). Morphological and developmental descriptions combined with molecular and genomic analyses will allow the identification of genes or genomic regions involved in plant responses to environmental constraints. Results from these analyses will be used to develop guidelines for genetic improvement of these vegetables to face climate change. The description of this new diversity will also be a key point to promote endangered local landraces and to promote the most suitable local landraces.

The BrasExplor project will provide a better understanding of the genetic determinism of adaptation to climatic variations which these crop species are exposed to. Valuable information on the genetic basis of such adaptation and comprehensive data on why certain genotypes are tolerant or resilient to the environmental constraints will lead to genetic improvement in view of climatic changes.

ACKNOWLEDGMENTS

We are grateful to Benjamin Foltran and Fulvia Rizza for their contribution to the project, Anne-Sophie Grenier for her input in communication and project website. We also thank Hakima Arrar, Fariza Boussad, Thouraya Rhim, Ilahy Riadh who are involved in field phenotyping. BrasExplor project is funded by the PRIMA programme supported under Horizon 2020 the European Union's framework programme for research and innovation.

Literature cited

- Aissiou F., Laperche A., Falentin C., Lodé M., Deniot G., Boutet G., Régnier F., Trotoux G., Huteau V., Coriton O., Rousseau-Gueutin M., Abrous O., Chèvre A. M., Hadj-Arab H. (2018). A novel *Brassica rapa* L. genetic diversity found in Algeria. *Euphytica* (2018) 214-241. <https://doi.org/10.1007/s10681-018-2318-9>
- Baskin and Baskin, 1998. *Seeds. Ecology, Biogeography and evolution of dormancy and germination*. US: Academic Press, San Diego, pp 666.
- Beacham, A.M., Hand, P., Pink, D.A., and Monaghan, J.M. (2017). Analysis of *Brassica oleracea* early stage abiotic stress responses reveals tolerance in multiple crop types and for multiple sources of stress. *J. Sci. Food Agric.* 97, 5271-5277.
- Bird, K.A., An, H., Gazave, E., Gore, M.A., Pires, J.C., Robertson, L.D., and Labate, J.A. (2017). Population Structure and Phylogenetic Relationships in a Diverse Panel of *Brassica rapa* L. *Front Plant Sci* 8, 321.
- Castillo-Lorenzo E., Finch-Savage W.E., Seal C.E., Pritchard H.W. (2019). Adaptive significance of functional germination traits in crop wild relatives of *Brassica*. *Agricultural and Forest Meteorology* 264 (2019) 343-350.

- Cheng, F., Sun, R., Hou, X., Zheng, H., Zhang, F., Zhang, Y., Liu, B., Liang, J., Zhuang, M., Liu, Y., et al. (2016). Subgenome parallel selection is associated with morphotype diversification and convergent crop domestication in *Brassica rapa* and *Brassica oleracea*. *Nat. Genet.* *48*, 1218–1224.
- De Jong, T.J., Isanta, M.T., and Hesse, E. (2013). Comparison of the crop species *Brassica napus* and wild *B. rapa*: characteristics relevant for building up a persistent seed bank in the soil. *Seed Science Research* *23*, 169–179.
- Demilly D., Ducournau S., Wagner M.H., and Dürr C., 2014. Digital imaging of seed germination. In S. Dutta Gupta and Y.Ibaraki (eds.) *Plant Image Analysis: Fundamentals and Applications*. CRC Press, chapter 7: 147-162.
- Finch-Savage, W.E., Clay, H.A., Lynn, J.R., and Morris, K. (2010). Towards a genetic understanding of seed vigour in small-seeded crops using natural variation in *Brassica oleracea*. *Plant Science* *179*, 582–589.
- Gardarin, A., Coste, F., Wagner, M.-H., and Dürr, C. (2016). How do seed and seedling traits influence germination and emergence parameters in crop species? A comparative analysis. *Seed Science Research* *26*, 317–331.
- Hatzig S., Breuer F, Nesi N, Ducournau S, Wagner M-H., Leckband G., Abbadi A. and Snowdon RJ, 2018. Hidden effects of seed quality breeding on germination in oilseed rape (*Brassica napus* L.). *Front. Plant Sci.* *9*:419. doi: 10.3389/fpls.2018.00419.
- Qi, X., An, H., Ragsdale, A.P., Hall, T.E., Gutenkunst, R.N., Pires, J.C., and Barker, M.S. (2017). Genomic inferences of domestication events are corroborated by written records in *Brassica rapa*. *Molecular Ecology* *26*, 3373–3388.