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SolACE - Solutions for improving Agroecosystem and Crop Efficiency for water and nutrient use

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Wheat and potato gene-based markers

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1. Introduction

The development of the root is a key element for plants to face abiotic stress, and especially those related to restricted availability of below-ground resources (water or nutrient shortage). The characteristics of the root system can indeed make more nutrient or water available to the plant at key stages. It was for example shown that varieties with deeper root system could be more productive in drought conditions (Manschadi et al. 2006 ; Ehdaie et al. 2012). However, these traits have rarely been selected directly by plant breeders, because of the difficulty to phenotype below-ground traits and of their interaction with the environment. Therefore, the SolACE project aims at associating genetic variants of candidate genes and other genomic markers to phenotypic variation of root traits to obtain indirect selection tools for such traits still difficult to phenotype in practical breeding, and to select for by breeders.

BREAD AND DURUM WHEAT. The use of high-throughput phenotyping platforms such as those developed by INRAE (4PMI) and UCL (RootPhAir) can be used to characterize intensively panels of diversity at early stages for root traits. This was done successfully within SolACE for bread and durum wheat. It revealed important genetic variabilities for all traits in both bread and durum wheat. The phenotypic data collected within SolACE could thus be combined with genotypic data available from previous projects to apply association mapping and genomic selection (GS) strategies.

This deliverable of WP4 (Novel Breeding Strategies and Tools) is related to the gene-based Marker loci identified for non-obvious below-ground traits of wheat, *i.e.* to the identification of the SNP marker loci that are genetically associated to the below-ground (root) traits segregating in the bread wheat and durum wheat panels. Reaching this deliverable means that we have been able to characterize genotypically and phenotypically the panels of wheat diversity, and that we have been able to phenotype them also for non-obvious traits linked to their adaptation to nitrogen and water shortage, such as traits correlated to root biomass, root depth and angle. Moreover, it means that we have verified that a sufficient variation exists within the two diversity panels for these traits, besides what was already shown for yield-related traits.

In parallel with the calibration of GS models (MS28), association mapping was carried out on the root traits to identify potential QTLs of interest. These traits are expected to be polygenic, but it is still interesting to check if some regions of the genome have a significant effect that could be used in breeding. This strategy of marker-assisted selection has already been successfully applied to rice for root traits related to drought resistance (Uga et al. 2013).

POTATO. Due to climate change and decrease in precipitations, it will become more important to breed for crops that are tolerant to dry and nutrient limited conditions. Although potato is a relatively water efficient crop compared to other staple crops, potato plants are sensitive to drought stress during the growing period. Especially drought during the tuberization period can lead to fewer and smaller tubers as well as to tuber malformations. Under drought stress, plants can reduce their water loss through leaves by closing stomata, but they can also increase their water and nutrient uptake by growing deeper roots. Drought tolerant phenotypes can be found in wild relatives of potato. By identifying functional traits for drought tolerance and developing markers for these traits, it will be possible to select dedicatedly for drought tolerant potato cultivars. The development of diploid hybrid potato has paved the way for application of genetic research to applied breeding programs (Lindhout et al., 2011; Stockem et al., 2020).

This study was set up to select traits that contribute to drought tolerance and to identify gene-based markers that are responsible for this. An F2 population was developed from a cross between a diploid *S. tuberosum var. phureja* cultivar named 'Andean Sunside' (Parent A) and a Solynta BC1F6 line (Parent B) derived originally from a cross between an S6 line (Phumichai et al. 2005) and a *S. tuberosum* diploid line. The homozygosity

percentages of the parents were calculated to be 75.48% and 64.97%, respectively. After the cross, one F1 plant (16HP5034-0016) was selected and selfed to collect the F2 seeds. This F2 population was used to perform a greenhouse pot experiment in which plant performance under drought was examined.

Reaching this deliverable of SolACE project means that we have been able to characterize genotypically and phenotypically the genetic populations created *ad hoc* for the project, and to phenotype them also for non-obvious traits linked to their adaptation to water shortage. Moreover, it means that we have verified that a sufficient variation exists in diploid potato for this trait, besides what was already shown for yield-related traits, and that on this variation an F1 hybrid breeding program can be continued.

2. Results

2.1 BREAD AND DURUM WHEAT

2.1.1 Distribution and heritabilities of the root traits

Important genetic variabilities were observed for all measured root traits. The heritabilities were high and always above 0.61. For both bread and durum wheat, the heritabilities, together with adjusted means and variances, are reported in Table 1. This shows that the adjusted means are reliable and that an important genetic variance is present in these panels. This is promising for the association mapping and genomic selection approaches to be implemented in SolACE.

Table 1: Distribution and heritabilities of the root traits measured in phenotypic platform for bread and durum wheat.

	Bread Wheat		
	Mean	Variance	Heritability
Aerial biomass at harvest (g/plant)	0.28	0.00255	0.75
Root biomass at harvest (g/plant)	0.2	0.00083	0.66
Number of seminal roots	4.3	0.32	0.76
Seminal root angle (°)	56	22.2	0.61
Root depth (mm)	278	1025	0.73
Width of the root bounding box (mm)	113	1188	0.55
Surface of the root convex hull (mm²)	21770	$5.27\ 10^7$	0.61
Root projected area (mm²)	1630	$1.64\ 10^5$	0.74

	Durum Wheat		
	Mean	Variance	Heritability
Aerial biomass at harvest (g/plant)	0.17	0.000822	0.68
Root biomass at harvest (g/plant)	0.13	0.000190	0.66
Number of seminal roots	4.1	0.29	0.68
Seminal root angle (°)	52	19.8	0.62
Root depth (mm)	287	2336	0.81
Width of the root bounding box (mm)	106	1646	0.66
Surface of the root convex hull (mm²)	21439	$8.03\ 10^7$	0.67
Root projected area (mm²)	1654	$2.52\ 10^5$	0.68

2.1.2 QTLs and gene-based markers

QTL analysis was performed to identify the genomic regions that contribute to the variability of the root traits measured in high-throughput phenotyping platform (INRAE-Agroécologie Dijon 4PMI and UCL RootPhAir). For bread wheat, SNPs were found significantly associated to the number of seminal roots, seminal root angle, and root biomass at harvest (Table 2). They are all located on chromosome 6B. For durum wheat, SNPs were found significantly associated to the aerial biomass at harvest. Whereas for the traits root depth (height of the root bounding box), width (width of the root bounding box), surface (surface of the root convex hull) of the box, including all root pixels at day 9 or 10, and for root area at day 9 or 10 (root projected area), SNPs were found significantly associated to the root depth and the root projected area only. These SNPs were significant even after a Bonferroni correction to take multiple testing into account.

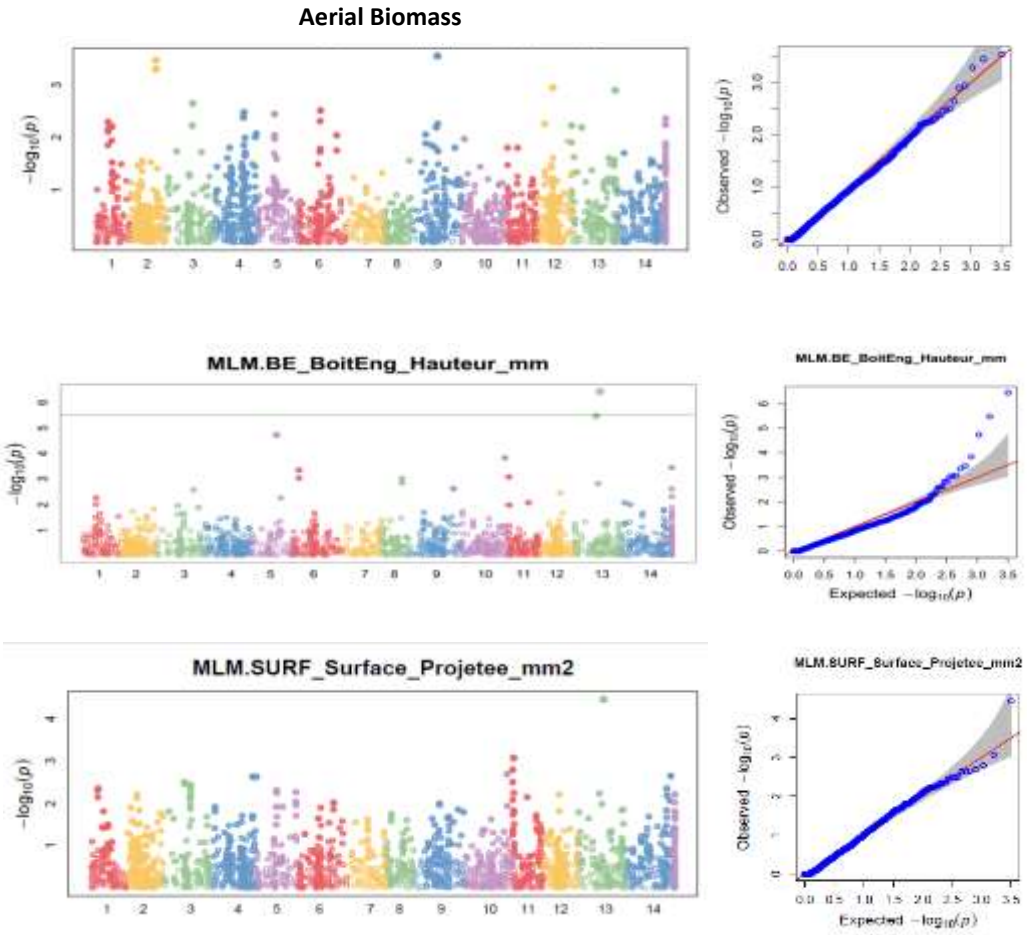
Table 2: List of QTLs (SNPs) associated to root traits and aerial biomass for bread wheat and the proportion of variance explained by each SNP for each trait. Significant associations after a Bonferroni correction are indicated in bold. Genetic positions rely on the "Chinese Spring x Renan" map.

			R2			
SNP_ID	**Chr.**	**Genetic position (cM)**	**Number of seminal roots**	**Seminal Root angle**	**Aerial Biomass at harvest**	**Root biomass at harvest**
cfn2961141	6B	61.302	**0.034**	0.007	0.001	0.001
cfn0019621	6B	88.866	0.001	**0.032**	0.001	0.002
cfn0102832	6B	88.935	0.001	**0.033**	0.002	0.002
cfn0145622	6B	88.866	0.001	**0.032**	0.001	0.001
cfn0851185	6B	88.992	0.001	**0.034**	0.001	0.001
cfn0852031	6B	88.866	0.002	**0.038**	0.002	0.002
cfn0860212	6B	88.992	0.001	**0.033**	0.001	0.001
cfn0860214	6B	88.992	0.001	**0.034**	0.001	0.001
cfn2980533	6B	88.866	0.001	**0.034**	0.002	0.002
cfn3041054	6B	88.866	0.001	**0.033**	0.001	0.002
cfn3052796	6B	88.866	0.001	**0.032**	0.001	0.001
cfn3006184	6B	61.043	0.020	0.001	0.021	**0.033**

Table 3: List of QTLs (SNPs) associated to root traits and aerial biomass for durum wheat, and the proportion of variance explained by each SNP for each trait. Genetic positions rely on the durum wheat consensus map (Maccaferri et al. 2015).

Trait	**SNP_ID**	**Chr.**	**Genetic Position (cM)**	**P-value**	**R2**	**Effect**
Aerial Biomass	IWB37548	5A	110.9	2.88E-04	0.095	0.073
Aerial Biomass	IWA5915	1B	115.8	3.51E-04	0.093	0.019
Root depth	IWB58970	7A	107.6	3.66E-07	0.180	-49.98
Root depth	IWB47576	7A	90.9	3.35E-06	0.159	40.00
Root depth	IWB49970	3A	114.3	1.87E-05	0.144	72.96
Root depth	IWA3359	5B	189.8	1.47E-04	0.127	-46.91
Root Projected area	IWB58970	7A	107.6	3.52E-05	0.081	-435.14

Figure 1: Manhattan and Q-Q plots of the association mapping analysis of two root traits (root depth ("Hauteur") and projected surface area ("Surface Projetee") and aerial biomass in durum wheat



In Tables 4 and 5 are indicated the closest genes to each QTL. This is not necessarily the gene responsible of the association, but it is the most probable. For bread wheat, the physical position of the SNPs on the Chinese Spring reference map was used to identify the closest annotated (IWGSC v1.0) genes.

Table 4: Gene-based markers identified in bread wheat, underlying the SNPs significantly associated to the below-ground traits using the IWGSC v1.0 annotation

Bread Wheat Trait	SNP_ID	Putative Function
Root biomass at harvest	cfn3006184	Myosin heavy chain-like protein, putative
Number of seminal roots	cfn2961141	Carboxypeptidase
Seminal root angle	cfn3052796	DNA binding protein, putative

Table 5: Gene-based markers identified in durum wheat, underlying the SNPs significantly associated to the below-ground traits using the Svevo reference genome annotation

Durum Wheat TRAIT	SNP_ID	Putative Function
Aerial Biomass	IWB37548	Glucosamine-6-phosphate deaminase
Aerial Biomass	IWA5915	Beta-1; 3-galactosyltransferase-like protein
Root depth	IWB58970	ATP synthase subunit beta
Root depth	IWB47576	10 kDa chaperonin
Root depth	IWB49970	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
Root depth	IWA3359	myb-like protein X
Root Projected area	IWB58970	ATP synthase subunit beta

For durum wheat, the physical position of the SNPs on the Svevo reference genome was used to identify the closest annotated genes.

The small number of significant SNP and their reduced effects on the trait, particularly in bread wheat, reveal that these traits are polygenic. In addition to this, we could not find any co-localization with yield QTL. This means that the SNPs that we have identified would be difficult to use in practice for breeding, but genomic prediction approaches will be useful.

2.2 POTATO

2.2.1 Plant performance

Plant performance was monitored during the experiment, starting before the start of the drought treatment. Weekly leaf appearance counts and biweekly plant height measurements (starting at 35 DAP) revealed a decline in plant growth with the start of the drought period (data not shown). For both vertical plant growth and leaf appearance this growth reduction was highly significant between genotypes ($p<0.001$).

Table 6*:* F2 population mean values for all measured and calculated trait parameters, P- values for analysis of variance for the tested traits and heritability of the traits (under the assumption that all differences between genotypes are genetic).

Parameter	Mean value	P value (one-way ANOVA)		Heritability
		Genotype	Block	
Tuber number	5.8	<0.001	<0.001	73%
sqrt of Tuber weight (g)	5.7	<0.001	<0.001	95%
sqrt of Average Tuberweight	1.0	<0.001	<0.001	77%
Visual wilting score (0-5)	1.2	<0.001	0.018	65%
Final plant height	57.9	<0.001	0.002	89%
Vertical plant growth during drought period	15.0	<0.001	0.136	73%
Natural logarithm (Vertical plant growth during drought period / water supplied during drought period)	0.0	<0.001	0.707	68%
Residual growth (PHvsVWC)	1.4	<0.001	0.203	80%
Leaf appearance during drought period	12.8	<0.001	0.805	68%

Final leaf number	41.4	<0.001	0.346	69%
Water supplied during drought period squared	1001.8	<0.001	0.216	56%
Natural logarithm (Leaf appearance during drought period / water supplied during drought period)	0.2	<0.001	0.992	75%
Plant fresh weight	59.2	<0.001	0.004	80%
Plant dry weight	7.0	<0.001	<0.001	77%
Plant dry weight / Plant fresh weight	0.1	<0.001	0.141	81%
Leaf RWC%	0.7	<0.001	<0.001	44%
Residuals of observed RWC% - model estimate (based on VWC%)	0.0	0.015	<0.001	33%
Average leaf temperature	23.6	0.842	<0.001	0%

Several additional growth parameters were measured (Table 6, Table 7), and analysis of variance revealed that except for leaf temperature, all parameters values were found to be significantly genotype-dependant (Table 7). Broad sense heritability percentages were calculated under the assumption that all differences between genotypes are genetic.

Table 6: F2 population mean values for all measured and calculated trait parameters, P- values for analysis of variance for the tested traits and heritability of the traits (under the assumption that all differences between genotypes are genetic).

Parameter	Mean value	P value (one-way ANOVA)		Heritability
		Genotype	Block	
Tuber number	5.8	<0.001	<0.001	73%
sqrt of Tuber weight (g)	5.7	<0.001	<0.001	95%
sqrt of Average Tuberweight	1.0	<0.001	<0.001	77%
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Leaf RWC%	0.7	<0.001	<0.001	44%
Residuals of observed RWC% - model estimate (based on VWC%)	0.0	0.015	<0.001	33%
Average leaf temperature	23.6	0.842	<0.001	0%

2.2.2 QTLs and gene-based markers

QTL analysis was performed to identify the genomic regions that contribute to the plant performance under drought of different physiological, growth and yield traits. For 6 out of 16 tested parameters significant major QTLs were found using the interval mapping approach. QTLs were identified on the chromosomes 3, 7, 10 and 12 (Figure 2 and Table 8). On chromosome 3, a QTL for vertical growth WUE was identified.

On chromosome 7, two overlapping QTLs for two vertical plant growth related traits under drought conditions were identified. QTL analysis for drought tolerance by Anithakumari, *et al.* (2012) identified over 2 consecutive years a QTL for plant height under drought stress conditions on the same chromosome. QTLs for plant height under control conditions were not found on chromosome 7 in that study. Therefore, the vertical growth-related QTLs identified in this study are hypothesized to explain variance in plant size due to drought stress and not plant performance in general. On chromosome 10 a QTL for plant height was identified.

Six significant QTLs for plant performance under drought conditions were identified on 4 distinct genetic loci. All traits for which QTLs were identified were significantly correlated with plant yield (tuber number and/or tuber weight), except for residual vertical plant growth. The plant height QTL identified on chromosome 7 was confirmed as a drought tolerance QTL by Anithakumari, et al. (2012). For QTLs on chromosome 3 and 7, parent A was identified to have the favorable allele. Therefore, integration of these QTLs from parent A in the Solynta germplasm would be favourable in breeding for plant performance under drought.

Besides the QTLs that were found in this study, other genes have been found in potato that are related to drought tolerance (Aliche et al. 2019, Yang et al. 2019). Aliche et al.(2019) found a SNP marker associated with tuber traits under drought conditions. For the SNP marker PotVar0030768 on chromosome 3 an additive effect of allele dosage for marketable fractions of tuber number and fresh yield. The marker was found at position 55,657,256 bp on the scaffold PGSC0003DMB000000062 of Chromosome 3 of the potato genome. Also in the plant material used in this study markers were used close to this position: solcap 18507 and solcap snp c2 26454 (Figure 2).

In a study by Yang et al. (2019) a diploid potato population was created segregating for drought tolerance. A drought sensitive and a drought tolerant genotype were selected and grown under dry conditions. Yang et al. (2019) found that a gene (PGSC0003DMG400004020) encoding *zeaxanthin epoxidase (ZEP)* was upregulated in the tolerant genotype. ZEP is an important enzyme involved in ABA synthesis and plant tolerance to drought. Though the gene was not found in our QTL study, a marker could be made for the population that was used in our study (Annex 1). This marker will be used to select for contrasting haplotypes in a drought experiment.

Table 8. QTL characteristics for the identified QTLs, by trait. For each of the identified QTLs the LOD score(s) from the genome wide QTL scan; the 95% confidence interval in centimorgan; the narrow sense heritability and the mean parameter values for allelic variation on QTL level (A = allele parent A, B = allele parent B).

Trait	QTL name	chromosome	LOD score	95% CI (cM) (1-LOD support)	h^2	Means values for:		
						AA	AB	BB
Vertical growth WUE	phWUE	3	3.61	49 – 69	24%	-3.4	-3.8	-3.9
Residuals of modelled vertical growth vs VWC%	rGrowth	7	4.55	28 – 72	26%	-2.9	4.3	8.1
Final plant height	finalPH1	7	5.18	37 – 75	23%	47	54	63
	finalPH2	10	3.8	2 – 18	19%	43	58	58

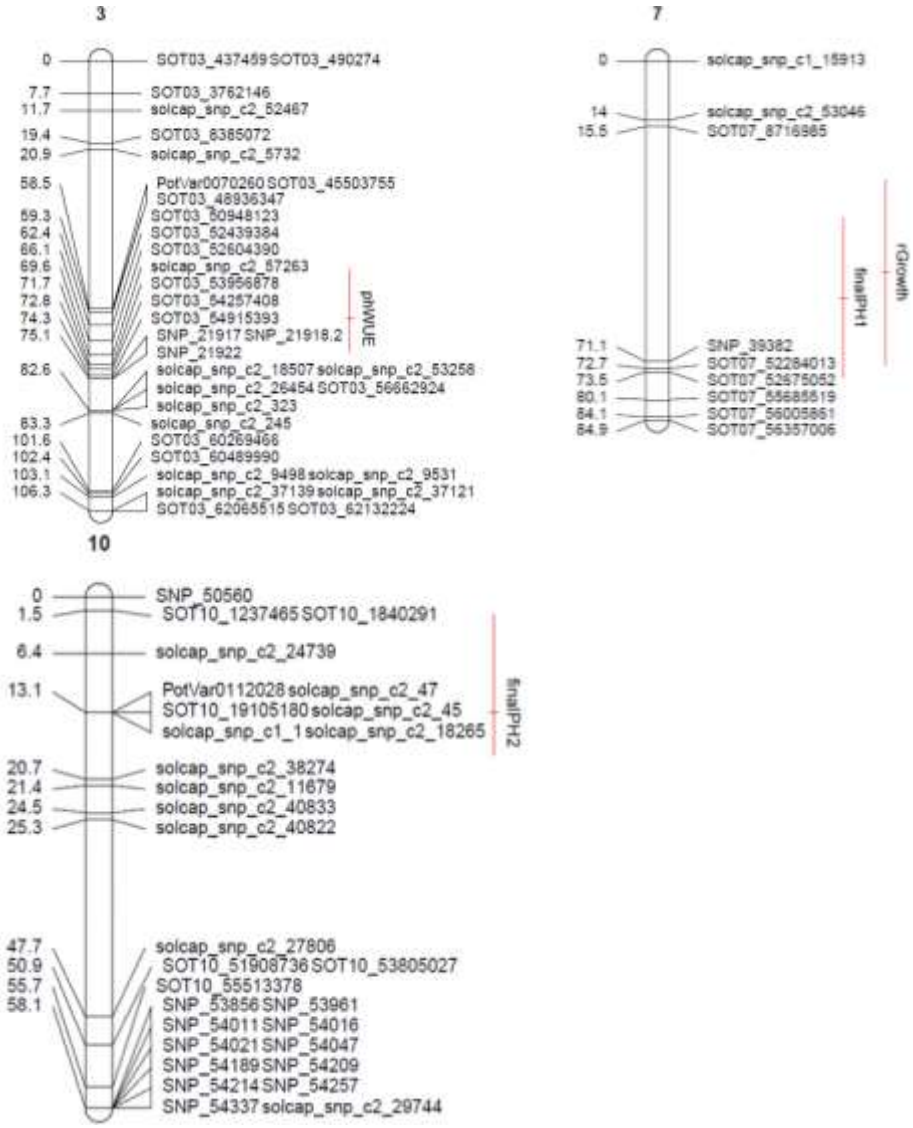

Figure 2 QTL Locations on the integrated genetic marker map. *Only the chromosomes on which QTLs were found are shown. Numbers on the left side represents genetic distance in centimorgans for the markers shown on the right side of each depicted chromosome. QTLs are drawn on the right side with red vertical lines as 95% confidence intervals. For QTL trait names and characteristics see Table 3.*

3. Conclusions

BREAD AND DURUM WHEAT. For bread wheat, 3 QTLs were found for root biomass at harvest, number of seminal roots and seminal root angle, with an effect explaining less than 3.8% of the phenotypic variance. They were all located on chromosome 6B and may be influenced by the same underlying genes.

For durum wheat using the SNP dataset of 3206 markers, seven QTLs were identified, of which five for root traits with the association mapping analysis on three chromosomes 7A, 3A and 5B, specifically for root depth and root projected area. The effects of the gene-based markers identified with the MLM model, with explained variances significantly higher than in bread wheat, reveal that besides a complex genetic basis of

the root traits there are loci for which It might be feasible to conduct a marker-assisted approach of introgression. Among the gene-based markers, interestingly a *myb-like* gene has been identified.

POTATO. For potato, six QTLs for drought tolerance were discovered. After validation in literature, at two locations a functional gene (encoding a zeaxanthin epoxidase) was found related to drought tolerance. At these sites, gene-based makers were developed, which were discovered in experiments and validated by recent literature. These SNP markers can be readily applied in a potato breeding program to select for drought tolerance.

4. Partners involved in the work

CREA, INRAE, Solynta

5. Annexes

Annex 1: Potato HRM marker alternative for solcap_snp_c2_27216

Primer_F
GAGCTTGGAAAATGGGCATA
Start: 19 Length: 20 bp Tm: 60.0 °C GC: 45.0 % ANY: 4.0 SELF: 2.0

Primer_R
CCCGCAACAAGTACTTTCAA
Start: 171 Length: 20 bp Tm: 58.8 °C GC: 45.0 % ANY: 8.0 SELF: 3.0

Annex 2: Literature cited

Aliche, Ernest B., Marian Oortwijn, Tom P.J.M. Theeuwen, Christian W.B. Bachem, Herman J. van Eck, Richard G.F. Visser, and C. Gerard van der Linden. 2019. "Genetic Mapping of Tuber Size Distribution and Marketable Tuber Yield under Drought Stress in Potatoes." *Euphytica* 215 (11).

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