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Impacts of heat and water stresses on the cultivated tomato *Solanum lycopersicum* and its wild relative *Solanum chilense*

Annexes

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Academic year 2021-2022

Master's Thesis submitted for the obtention of the degree of Agricultural Bioengineering

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Annex 1: DNA-testing the hybrids

1.1 Protocol

1.1.1 Extraction of tomato ADN

Protocol from Tanksley lab in Cornell, USA.

Preparation of the micro prep buffer :

5 mL extraction buffer

- 0.35 mol.L^{-1} sorbitol
- 0,1 mol.L^{-1} Tris-base
- 5. 10^{-3} mol.L^{-1} EDTA-Na_2
- Bring pH to 7.5 with HCl
- 0.02 mol.L^{-1} Na bisulfite

5 mL nuclei lysis buffer

- 0,2 mol.L^{-1} Tris
- 0.05 mol.L^{-1} EDTA-Na_2
- 2 mol.L^{-1} NaCl
- 2% CTAB

2 mL sarkosyl 5%

- 5 g sodium lauryol sarconisate in 100 mL

0.02 g sodium bisulfite

Procedure :

1) Grind tomato leaflets in liquid nitrogen, homogenize it in 750 µL micro prep buffer and let it incubate 30 to 60 min at 65°C.
2) Fill the tube with chloroform/isoamyl alcohol (24:1).
3) Centrifuge 5 min at 9595*g* and collect the supernatant in another tube.
4) Add 1 volume of isopropanol and reverse the tube until the DNA precipitates.
5) Wash the residue with ethanol 70% then let it dry in the open tube.
6) Re-suspend the DNA in 50 µL sterile milli-Q water and let it 30 to 60 min at 65°C.
7) Centrifugate 10 min at 9595*g* to precipitate starch.
8) Gather 3µL of supernatant.

1.1.2 Polymerase chain reaction (PCR)

The markers that are going to be used are:

Marker	Chromo some	cM (Tomato -EXPEN 2000)	Annealing tempe- rature	*Restriction enzyme*	Fragment size		Primer sequence (5'-3')
					S. lyco- persicum	*S. chilense*	
C2_At4g04955	2	63.5	55	*HinfI*	400	300	TTGCTGTGGGGAACCAAGCAGATATAG TCCCAGAGAGTCTTGATCCCATGTATGC
C2_At1g28530	3	21	55	*DraI*	600	400	ATTATGAAGATGTCTATACACTTCCCTAC AGAGATTGCTTTTGACATAGAAATGCTT
C2At3g08760	10	39	55	*AluI*	500/600	400	TCTCCAGAACGTTGTGTGTCAGAAGG TCCTCATGTAGAAATGTAAGACCTTG
T0386A	11	85	52	*HinfI*	500	200/300	ATGCTGATGAAAGATTGGGCGCTG TTAGGCTTTGGCTTCTCGACCACT

For each marker, prepare the following mix :

Substance	Volume [µL]
H_2O	15.375
Buffer + $MgCl_2$	5
dNTP (20 mmol/L)	0.5
Primer R (10 µmol/L)	0.5
Primer F (10 µmol/L)	0.5
GoTaq buffer (5 units/µL)	0.125
DNA (0.1x)	3

Heat these 25 µL samples at 94°C for 2 minutes then apply the following cycle 39 times : 94°C (30"), 55°C (30") and 72°C (5 minutes). Once the 39 cycles are done, heat the samples at 72°C for 5 minutes. After amplification, add 0.1 µL of the restriction enzyme corresponding to the marker and 1.25 µL of buffer to 10 µL of PCR mix and 1.2 µL of water. Let it incubate overnight at 37°C. The samples can then be put on a 1% agarose gel and migrated.

1.2 Results

The tested individuals were supposed to be hybrids. In this PCR test, hybrids should present both bands from *S. lycopersicum* and *S. chilense*. The tested individuals did not present the bands from *S. chilense* but presented the same bands as those of *S. lycopersicum* (Figure 1).

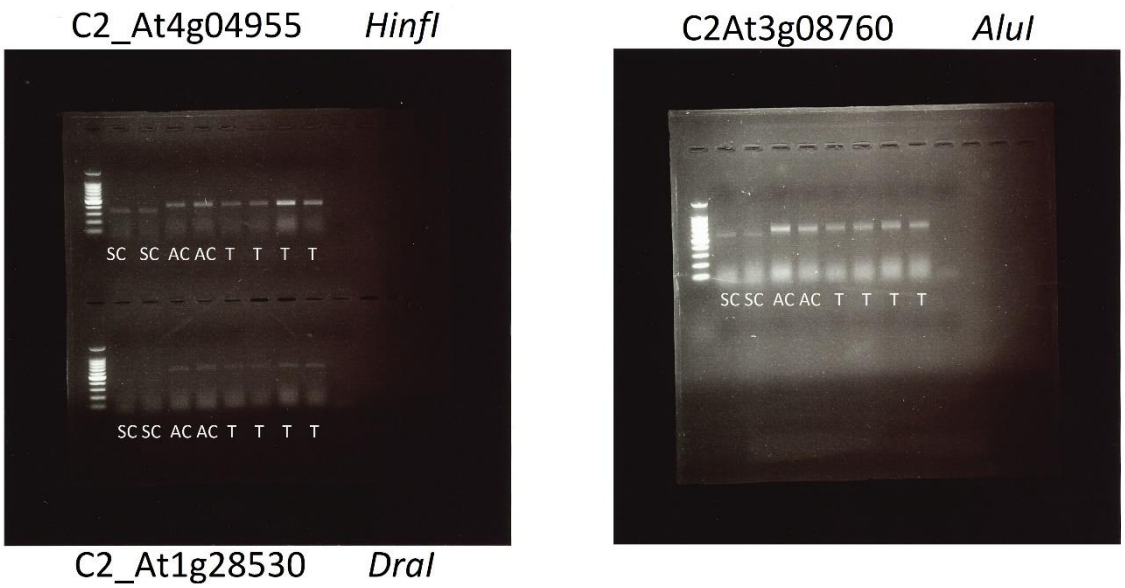

Figure 1 – Picture of the results from the PCR test. AC = S. lycopersicum ; *SC =* S. chilense ; *T = tested individuals. The name of the corresponding marker and the restriction enzyme are shown.*

1.3 Conclusion

The tested individuals were not hybrids but actually *S. lycopersicum*. They were therefore not included in the analyses of the results.

Annex 2: Protocol for the determination of PEG content

D'après Boulter & McMichael, 1970; Lawlor, 1970

Gamme

Préparer une solution aqueuse de PEG 6000 à 0,1875 g/L = 187,5 mg/L.
Diluer la solution comme suit pour préparer la gamme. Prévoir 3 réplicats.

mg_{PEG}/g_{MF}	0	0,5	1	1,5	2	2,5	3	3,5	4	4,5	5
$V_{sol.PEG}$ [ml]	0	0,25	0,5	0,75	1	1,25	1,5	1,75	2	2,25	2,5
V_{eau} [ml]	3	2,75	2,5	2,25	2	1,75	1,5	1,25	1	0,75	0,5

Solutions à préparer

- À l'avance :
    - **$BaCl_2$** 10% (A47)
    - **$Ba(OH)_2$** AR 0,3N soit 0.15 mol/L ou 47,322 g/L (A328)
    - **$ZnSO_4$** 5% (A2)
    - **TCA** (acide trichloracétique) 30% (F53) avec **$BaCl_2$** 5% (A47)
    - **Gomme arabique** 36 mg/L (M226)
- Le jour même :
    - **Mélange TCA** constitué de 4 parts de TCA+$BaCl_2$ et 1 part de gomme arabique.

Protocole

- Séparer les différentes parties de la plante (systèmes racinaire et aérien).
- Broyer environ 500 mg d'échantillon frais à l'azote liquide (peser échantillon).
- Suspendre dans 8 mL d'**eau distillée.**
- Centrifuger 10 minutes à 500G pour enlever les débris et filtrer.
- Amener la solution filtrée à 10 mL à l'**eau distillée.**
- Précipiter les protéines avec les réactifs de Somogyi (à ajouter dans l'ordre) :
    - 3 mL de la **solution** échantillonnée (utiliser les 10 mL pour faire 3 réplicats)
    - 3 mL d'**eau distillée**
    - 0,6 mL de **$BaCl_2$** 10%
    - 1,2 mL de **$Ba(OH)_2$** AR 0,3N
    - 1,2 mL de **$ZnSO_4$** 5%

Faire de même avec les 3 réplicats de la gamme.

- Centrifuger 10 minutes à 500G et récupérer 2 mL de surnageant.
- Ajouter 2 mL du **mélange TCA** aux 2 mL de **surnageant** pour précipiter les protéines et acides nucléiques restants.
- Mesurer la transmission après 5 minutes à 420 et 635 nm.

Annex 3: Statistical results from experiment 1

This table shows the results of standard analysis of variance (F statistics) or Kruskal-Wallis tests (χ^2 statistics) on data from experiment 1. Significant factors are showed in bold. Only significant interactions were put in the table in order to keep it lighter.

The confidence level was set to 0.95 and sometimes lowered to 0.90 if some effects were almost significant at 0.95.

PARAMETER	FACTOR	TEST STATISTIC	P-VALUE
Biomass production	**Species**	**F = 69.6159**	**$3.20*10^{-7}$**
	Temperature	F = 1.8513	0.1925
	Organ	**F = 68.7569**	**$3.47*10^{-7}$**
	Species:Part	**F = 38.1467**	**$1.33*10^{-5}$**
Number of leaves at the end of experiment	**Species**	**χ^2 = 22.318**	**$2.31*10^{-6}$**
	Temperature	χ^2 = 0.020546	0.886
	Water	**χ^2 = 30.82**	**$2.831*10^{-8}$**
Leaf area	Species	F = 13.8761	0.005829
	Temperature	F = 0.4677	0.513373
Stomatal density	**Species**	**χ^2 = 32.976**	**$9.332*10^{-9}$**
	Temperature	**χ^2 = 5.7547**	**0.01644**
	Water	χ^2 = 1.3157	0.2514
Root length	**Species**	**χ^2 = 7.4034**	**0.00651**
	Temperature	**χ^2 = 9.6136**	**0.001931**
Water content	Species	F = 1.7073	0.2098
	Temperature	F = 0.159	0.69538
	Organ	**F = 52.2423**	**$2.02*10^{-6}$**
	Species:Organ	**F = 4.6162**	**0.04733**
CCI	**Species**	**χ^2 = 18.173**	**$2.017*10^{-5}$**
	Temperature	χ^2 = 1.9368	0.164
	Water	χ^2 = 14.769	0.0001215
Osmotic potential	Species	χ^2 = 2.7825	0.0953
	Temperature	χ^2 = 0.34018	0.5597
	Water	**χ^2 = 26.937**	**$2.102*10^{-7}$**
Malondialdehyde content	**Species**	**F = 12.1331**	**0.00828**
	Temperature	F = 0.1116	0.74694
Total soluble sugars content	**Species**	F = 3.7626	**0.06127**
	Temperature	F = 1.6595	0.20691
	Species:Temperature	**F = 3.9701**	**0.0549**
Photosynthesis at saturating irradiance	**Species**	**F = 15.3644**	**0.01725**
	Temperature	F = 0.3775	0.57218
	Water	F = 0.0154	0.90731
Maximum apparent yield of photosynthesis	Species	F = 0.1233	0.74316
	Temperature	F = 0.8384	0.41167
	Water	F = 5.9144	0.07183
Light Compensation Point	**Species**	**F = 7.8485**	**0.048736**

		Temperature	**F = 33.8818**	**0.004338**
		Water	**F = 54.314**	**0.001806**
PAR at 75% of saturation of photosynthesis		Species	F = 1.0555	0.3623
		Temperature	F = 0.1491	0.7191
		Water	F = 1.3728	0.3064
Electron transport rate at saturating irradiance		Species	F = 2.4105	0.1516
		Temperature	F = 0.0574	0.8155
		Water	F = 1.3795	0.2674
Minimum saturating irradiance		Species	χ^2 = 1.864	0.1722
		Temperature	χ^2 = 0.75294	0.3855
		Water	χ^2 = 0.54751	0.4593
Efficiency of photosystem II		Species	χ^2 = 3.333	0.06789
		Temperature	χ^2 = 0.3	0.5839
		Water	**χ^2 = 5.0417**	**0.02474**
Transpiration		Species	F = 5.8034	0.07363
		Temperature	F = 4.002	0.11605
		Water	**F = 11.9906**	**0.02575**
Stomatal conductance		Species	χ^2 = 3.1527	0.0758
		Temperature	χ^2 = 1.1364	0.2864
		Water	**χ^2 = 4.3636**	**0.03671**
Polyethylene glycol		**Wavelength**	**χ^2 = 37.568**	**$8.826*10^{-10}$**
	420 nm	Species	χ^2 = 1.528	0.2164
		Temperature	**χ^2 = 11.39**	**$7.386*10^{-4}$**
		Water	**χ^2 = 59.824**	**$1.037*10^{-14}$**
	635 nm	**Species**	**χ^2 = 6.8298**	**0.008965**
		Temperature	**χ^2 = 9.7435**	**0.0018**
		Water	**χ^2 = 52.127**	**$5.203*10^{-13}$**

Annex 4: Statistical results from experiment 2

This table shows the results of standard analysis of variance (F values) or Kruskal-Wallis tests (χ^2 values) on data from experiment 2. Significant factors are showed in bold. Only significant interactions were put in the table in order to keep it lighter.

The confidence level was set to 0.95 and sometimes lowered to 0.90 if some effects were almost significant at 0.95.

PARAMETER	FACTOR	TEST STATISTIC	P-VALUE
Biomass production	**Species**	**$\chi^2 = 21.51$**	**$3.519*10^{-6}$**
	Organ	**$\chi^2 = 6.9155$**	**0.0315**
	Water	**$\chi^2 = 26.564$**	**$2.55*10^{-7}$**
Number of leaves at the end of experiment	**Species**	**$\chi^2 = 92.719$**	**$<2.2*10^{-16}$**
	Water	**$\chi^2 = 47.905$**	**$4.474*10^{-12}$**
Leaf area	**Species**	**$F = 103.481$**	**$7.47*10^{-6}$**
	Water	**$F = 122.777$**	**$3.93*10^{-6}$**
	Species:Water	**$F = 93.107$**	**$1.11*10^{-5}$**
Stomatal density	**Species**	**$\chi^2 = 40.414$**	**$2.054*10^{-10}$**
	Water	$\chi^2 = 0.00497$	0.9438
Root length	**Species**	**$\chi^2 = 13.009$**	**$3.1*10^{-4}$**
	Water	**$\chi^2 = 12.333$**	**$4.449*10^{-4}$**
Root diameter	**Species**	**$F = 61.2953$**	**$8.64*10^{-12}$**
	Water	**$F = 272.7071$**	**$<1*10^{-16}$**
	Segment	**$F = 144.2315$**	**$<1*10^{-16}$**
	Species:Water	**$F = 66.4411$**	**$1.86*10^{-12}$**
	Treatment:Segment	**$F = 10.9638$**	**$5.42*10^{-5}$**
Xylem elements diameter	**Species**	**$\chi^2 = 27.859$**	**$1.305*10^{-7}$**
	Segment	**$\chi^2 = 18.456$**	**$1.005*10^{-3}$**
	Water	**$\chi^2 = 90.844$**	**$<2.2*10^{-16}$**
Ratio xylem elements diameter/root diameter	Species	$\chi^2 = 0.02027$	0.8868
	Segment	**$\chi^2 = 95.121$**	**$<2.2*10^{-16}$**
	Water	**$\chi^2 = 22.408$**	**$2.204*10^{-6}$**
Water content	**Species**	**5.5381**	**0.01861**
	Organ	**24.516**	**$4.748*10^{-6}$**
	Part	**14.743**	**1.232*10-4**
Chlorophyll content index	**Species**	**$F = 61.4481$**	**$7.21*10^{-7}$**
	Water	$F = 0.0572$	0.814
	Water:Species	**$F = 28.9884$**	**$6.08*10^{-5}$**
Osmotic potential	**Species**	**$F = 35.864$**	**$2.48*10^{-5}$**

Malondialdehyde content	Species	**χ^2 =** 2.0202	0.1552
	Organ	χ^2 = 3.0992	0.07833
	Water	**χ^2 = 3.9596**	**0.0466**
Total soluble sugars content	Species	F = 0.8126	0.3726
	Water	**F = 95.4087**	**$2.911*10^{-12}$**
	Organ	**F = 284.2863**	**$<2.2*10^{-16}$**
	Treatment:Organ	**F = 51.5927**	**$9.108*10^{-9}$**