Validation of two *Fusarium oxysporum* resistance-related SNPs in wild and cultivated beets

Background

Sugar beet

- Sugar beet (*Beta vulgaris* ssp. *vulgaris*) was domesticated from wild type sea beet (*B. vulgaris* ssp. *maritima*) (Biancardi et al. 2011)

- Production of sugar beet in temperate regions supplies about 20% of the world's sugar (Fao 2013)

Fusarium oxysporum

C. Rush

- Plant pathogenic fungi are the major biotic threats

- *Fusarium oxysporum* f. sp. *betea* (Fob) as the important disrupters cause two types of symptoms:
  - Fusarium yellows (Stewart 1931)
  - Fusarium root rots (Harveson 2009)

- The best management achieved by improving the plant inherent resistance

- Sugar beet breeders are trying to find the best resistant genes in donor parents to cope with this disease

Molecular markers

- Molecular markers facilitate the selection of resistant genes

- SNPs have found a special importance in association studies

- Using SNPs:
  – mapping the pathogen resistant genes in sugar beet (Stevanato et al. 2012; Brocanello et al. 2017; De Lucchi et al. 2017)

  – analyzing changes inside resistance genes analogues (RGAs) (Stevanato et al. 2017)

Fusarium-resistance SNPs
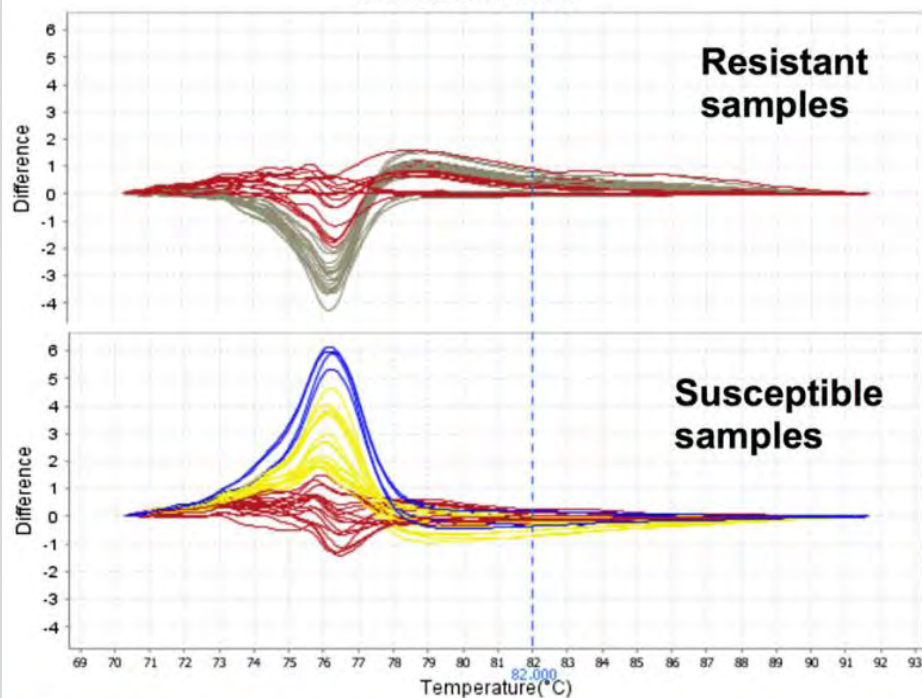
Wild type beet
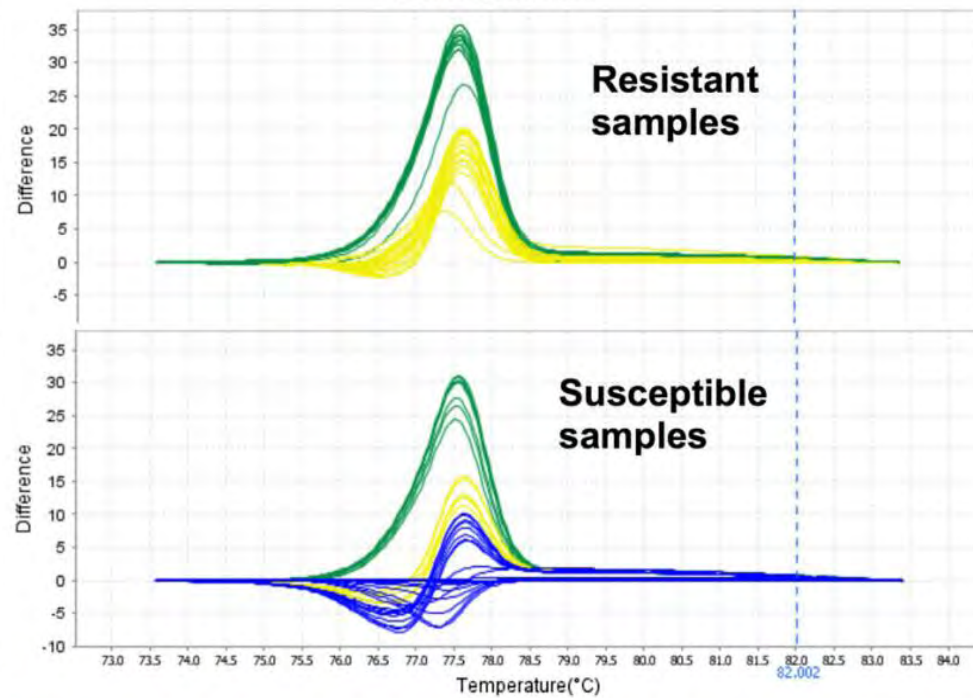
• Wild type plants are the best untapped genetic resources of resistance genes in breeding (Campbell 2010)

• First attempt to take advantage of the wild beet resistance-related genes began in the late 20th century

• Rhizoctonia root and crown rot, rhizomania, powdery mildew, cercospora leaf spot, aphanomyces root rot, and fusarium yellows resistance cultivars introgressed from wild beets using cross over (Campbell 2010)

Beta maritima.

Sugar beet wild relatives genotyping

- Wild beets genotyping using two Fob resistance SNPs help to:
  - Finding the resistant genotype inside these valuable species

  - Understanding the evolutionary process of Fusarium resistance genes

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  - Targeted application of the resistance genes

Wild beet genome screening

• In this study, different genotypes of wild beets (*B. maritima*) along with cultivated beets were screened for two available Fob-resistance SNPs

• This study is the first challenge in screening of wild sea beets genome for two new Fob-resistant SNPs

MaterialandMethodes

Plant material and Sampling location

- A collection of wild type seeds along with 31 hybrids of commercial cultivars seeds were selected for genotyping

- Wild beet seeds collected from 12 geographical areas in the coasts of the Adriatic Sea

- The commercial seed were provided by SBSI-sugar beet seed institute Karaj-Iran and DAFNAE-Department of Agronomy, Food, Natural resources, Animals and Environment (University of Padova, Italy)

Commercial cultivars inoculation

- The most pathogenic isolates of *F. oxysporum* f. sp. *betae* collected from symptomatic sugar beets in Iran was used for inoculation

- The effector genes of isolate was detected using 8 most important effector genes primers (Covey et al. 2014)

DNA isolation, Primer design and Genotyping

- BioSprint 96 platform (Qiagen, Germany) was applied following the methode defined by Stevanato et al. (2018)

- Surrounding sequences of two SNPs (De Lucchi et al. 2017) were used for primer design based on rhAmp assay

- The genotyping was performed following the procedure described from Broccanello et al. (2018) with QuantStudio 12 K Flex Real-Time PCR System

Statistical analysis

- SAS software was used

- ANOVA and Tukey HSD test were applied for AUDPC means variance analysis between genotypes at P<0.05

- Linear regression model was conducted for association studies between two SNPs and AUDPC

- Chi square (χ^2) test was used for calculation of allele frequencies of two SNPs in wild plant genotypes

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Results

Table 1. Response of sugar beet cultivars against *F. oxysporum* f. sp. *betae* isolate

Number	Cultivar ID	AUDPC	Number	Cultivar ID	AUDPC
7	Aria	525	4	Palma	1003.33
10	BTS213	694.16	20	Lorigout	1003.33
3	Ektbatan	787.5	12	DS4057	1026.66
11	Paya	863.33	28	Tokan	1055.83
21	Delta	880.83	6	Merak	1079.16
13	Dorothea	886.66	17	Linda	1085
9	Novodore	892.5	23	Perfekta	1096.66
25	Azare	915.83	16	Rosier	1102.5
31	Ukrain	921.66	5	Isabella	1137.5
2	Shokofa	945	22	Baloo	1155
24	Murialle	945	19	Ghazira	1190
14	BTS233	950.83	26	Antek	1213.33
15	Pars	950.83	27	Sharif	1260
1	Flores	968.33	29	Chimene	1376.66
8	Rajah	974.16	18	BTS335	1446.66
			30	Iris	1464.16

DSI investigation
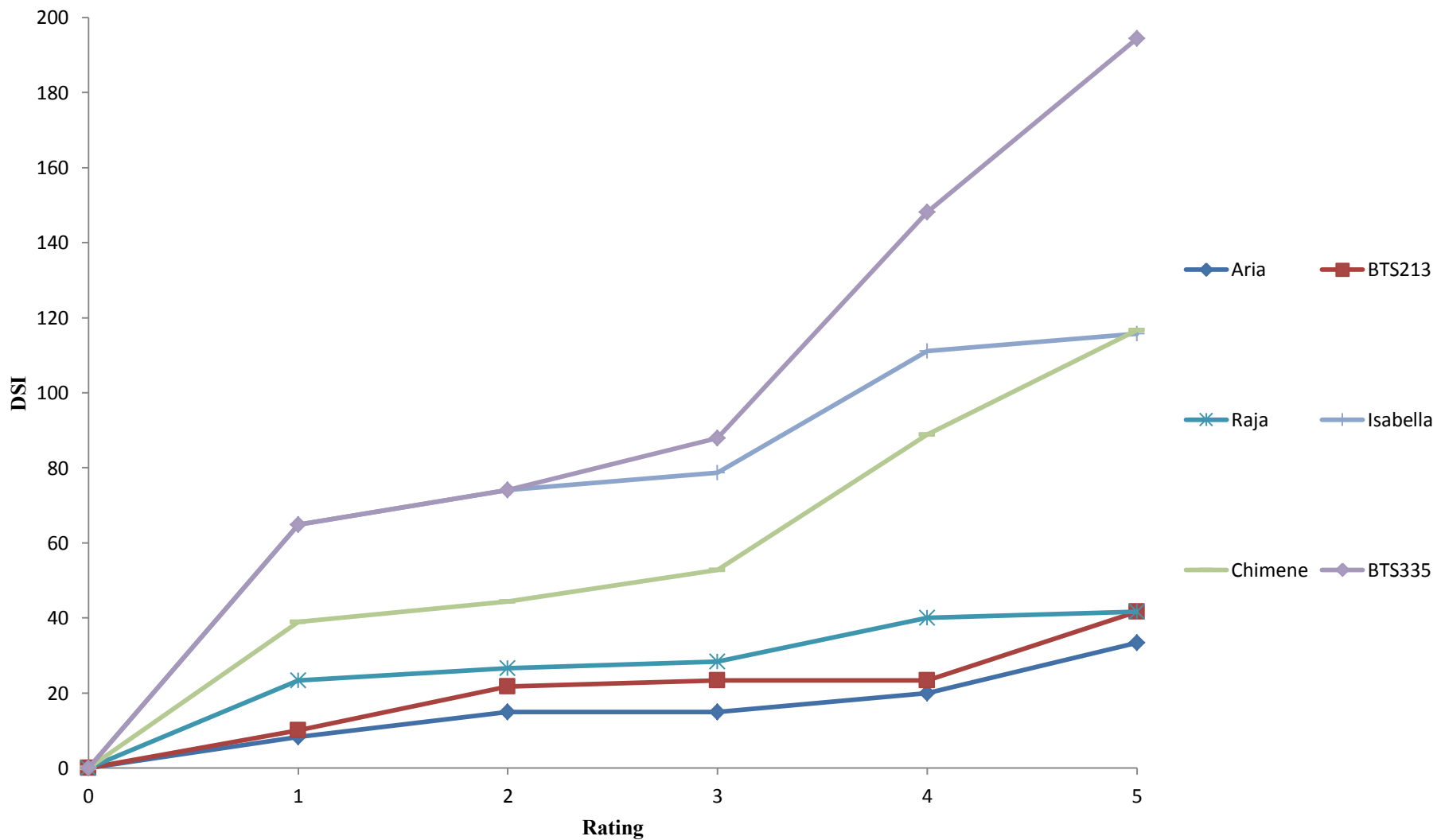
Genotyping and association results

- RhAmp assay was successfully able to genotype both the wild and the commercial varieties

- Both SNPs had a good degree of differentiation, but SNP1 showed high allele discrimination
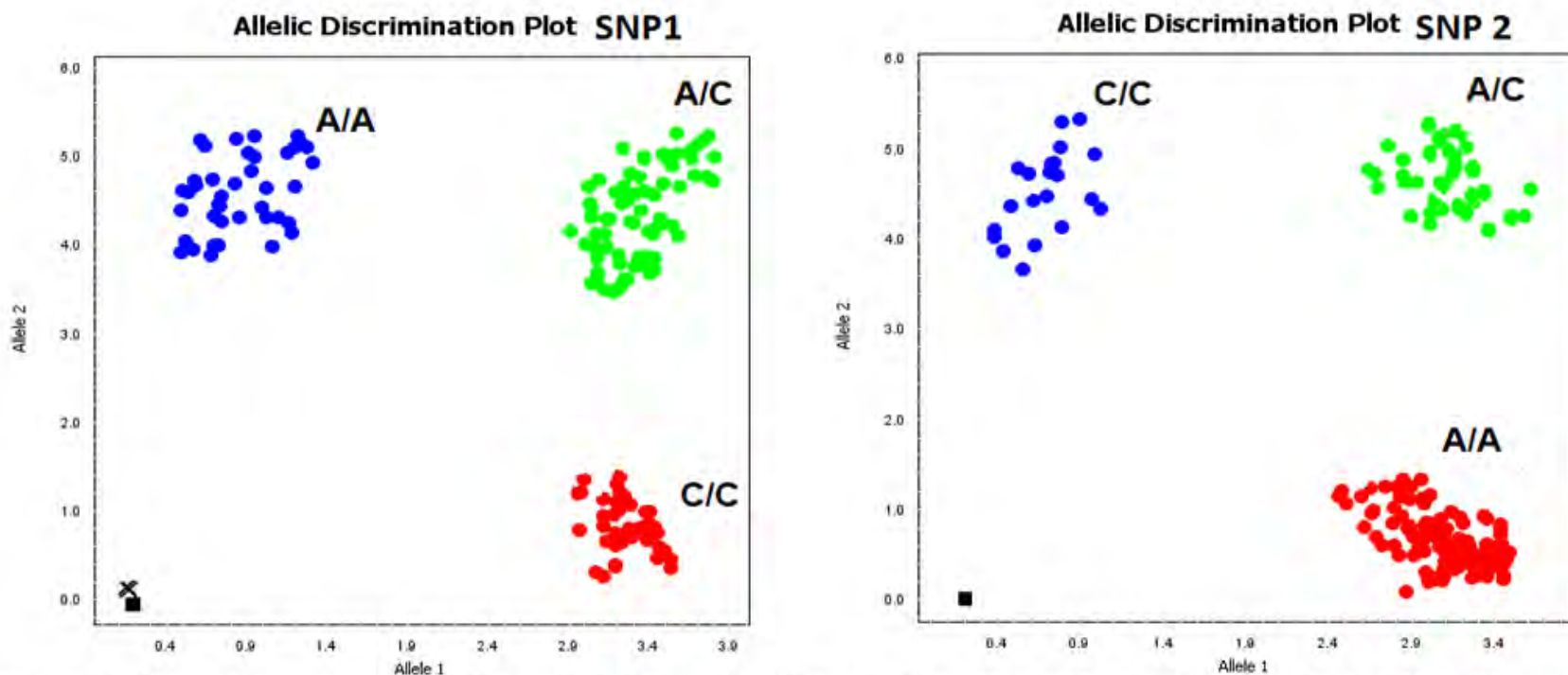

Fig 2. Allelic discrimination plots of SNP1 and SNP2 (SNP_Bv2_043450 and SNP_Bv7_171470) analysed by RH-AMP assay, the samples were involved 240 wild type species and 31 commercial cultivars.

Association between AUDPC and SNP genotyping
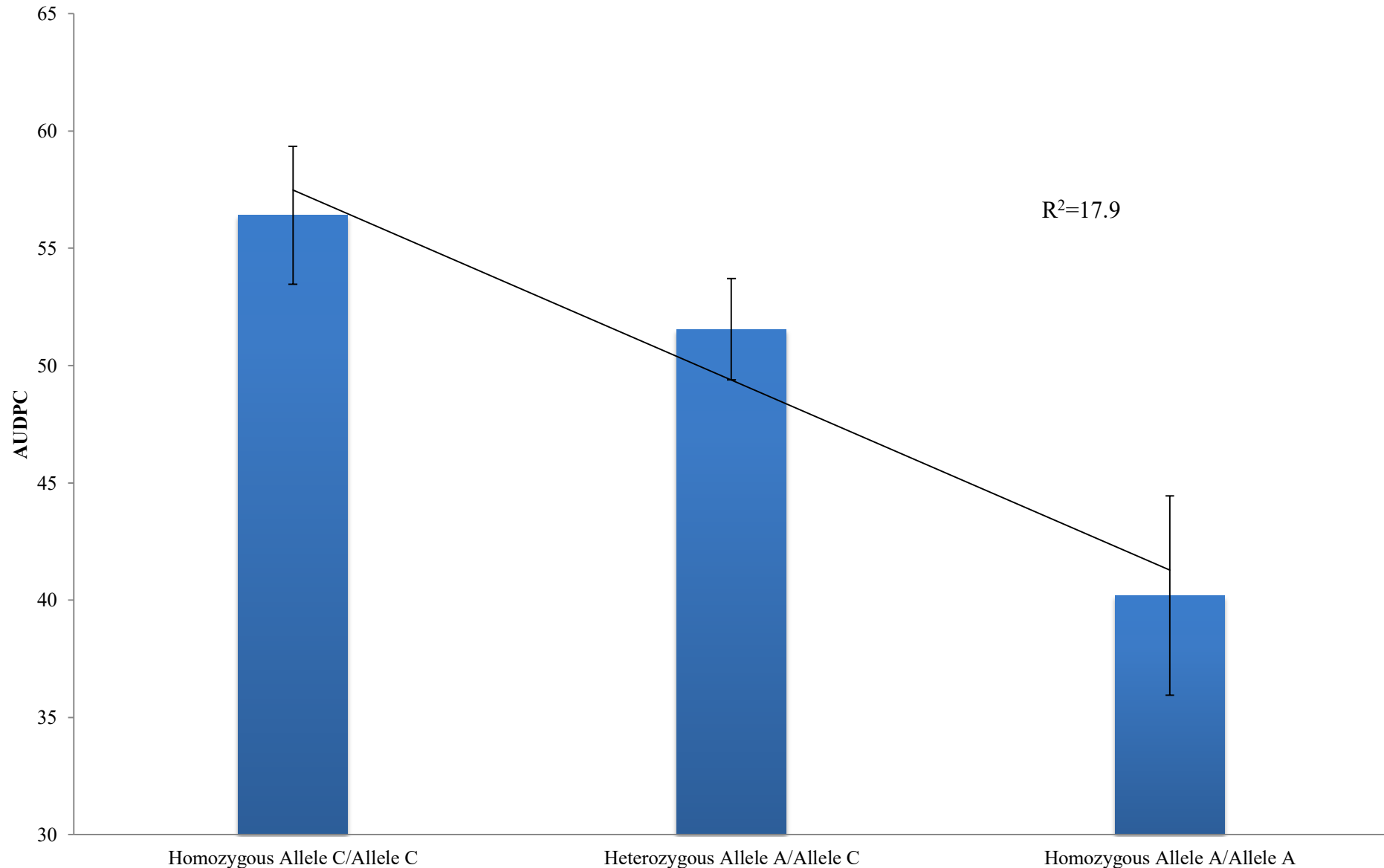
Coefficient correlation

Table 3. Determination Coefficient (R^2), probability value (P-value) and association score [-Log10(P-value)] SNP1 and SNP2 in sugar beet commercial cultivars

Model	R	R^2	P-value	-Log10(P-value)
SNP1	.423[a]	.179	0.018	1.744727
SNP2	.152[a]	0.023	0.415	0.381952

Table 5. Comparison of genotypes and alleles frequencies of two SNP1 and SNP2 between sugar beet wild relatives collected from some coasts of Adriatic Sea

ID		Frequency of SNP1genotypes (%)				Frequency of SNP2 genotypes (%)			
Allele		C	-	A	P value	C	-	A	P value
Genotypes		C/C	A/C	A/A		C/C	A/C	A/A	
Bm90	Alleles [n(%)]	9 (69.2)	-	4 (30.8)	.000	12 (85.5)	-	(2) 14.3	.000
	Genotypes [n(%)]	4 (57.1)	1 (14.3)	2 (28.6)		5 (71.4)	2 (28.6)	.0	
Rzm2	Alleles [n(%)]	8 (100)	-	.0	.000	.0	-	8 (100)	.000
	Genotypes [n(%)]	4 (100)	.0	.0		.0	.0	4 (100.0)	
Fm17	Alleles [n(%)]	6 (50)	-	6 (50)	.000	8 (66.7)	-	4 (33.3)	.000
	Genotypes [n(%)]	2 (33.3)	2 (33.3)	2 (33.3)		2 (33.3)	4 (66.7)	.0	
740	Alleles [n(%)]	6 (35.3)	-	11 (64.7)	.000	7 (43.8)	-	9 (56.2)	.000
	Genotypes [n(%)]	1 (12.5)	4 (50.0)	3 (37.5)		3 (37.5)	1 (12.5)	4 (50.0)	
Mgs	Alleles [n(%)]	13 (36.1)	-	23 (63.9)	.000	20 (76.9)	-	6 (23.1)	.000
	Genotypes [n(%)]	1 (7.7)	11 (84.6)	1 (7.7)		8 (61.5)	4 (30.8)	1 (7.7)	
Bocc	Alleles [n(%)]	14 (58.3)	-	10 (41.7)	.000	16 (53.3)	-	14 (46.7)	.000
	Genotypes [n(%)]	6 (42.9)	2 (14.3)	6 (42.9)		4 (26.7)	8 (53.3)	3 (20.0)	
Crv	Alleles [n(%)]	22 (20.4)	-	86 (79.6)	.000	18 (14.3)	-	108 (85.7)	.000
	Genotypes [n(%)]	.0	22 (34.4)	42 (65.6)		.0	18 (28.6)	45 (71.4)	
Ktr	Alleles [n(%)]	42 (56)	-	33 (44)	.000	21 (37.5)	-	35 (62.5)	.000
	Genotypes [n(%)]	13 (43.3)	16 (53.3)	1 (3.3)		2 (7.1)	17(60.7)	9 (32.1)	
Lstv	Alleles [n(%)]	42 (60)	-	28 (40)	.000	10 (16.1)	-	52 (83.9)	.000
	Genotypes [n(%)]	15 (48.4)	12 (38.7)	4 (12.9)		1 (3.2)	8 (25.8)	22 (71.0)	
Splt	Alleles [n(%)]	37 (46.8)	-	42 (53.2)	.000	2 (3)	-	64 (97)	.000
	Genotypes [n(%)]	9 (28.1)	19 (59.4)	4 (12.5)		.0	2 (6.1)	31 (93.9)	
Tml	Alleles [n(%)]	7 (28)	-	18 (72)	.000	4 (20)	-	16 (80)	.000
	Genotypes [n(%)]	.0	7 (63.6)	4 (36.4)		.0	4 (40.0)	6 (60.0)	
Vla	Alleles [n(%)]	12 (50)	-	12 (50)	.000	1 (5.6)	-	17 (94.4)	.000
	Genotypes [n(%)]	3 (33.3)	6 (66.7)	.0		.0	1 (11.1)	8 (88.9)	
Total	Alleles [n(%)]	218(44.4)	-	273(55.6)	.000	119 (26.2)	-	335 (73.8)	.000
	Genotypes [n(%)]	58 (25.3)	102(44.5)	69 (30.1)		25 (11.0)	69(30.4)	133 (58.6)	

Chi square test

- There was a significant difference between the observed genotype and the expected genotype

Table 6. Chi-Square Tests for Observed and Expected frequency of genotypes in both SNPs inside wild sugar beet

SNPs	SNP1			SNP2		
Genotypes	C/C	A/C	A/A	C/C	A/C	A/A
χ^2	2.38	72.2	17.1	78.08	175.5	23.01
P value	.000			.000		

Discussion

Differences of resistance-related SNPs

- Two types of Fob resistance-related SNPs were identified inside RGs (Sekhwal et al. 2015) of sugar beet genome (De Lucchi et al. 2017)

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  SNP1

  Bv2_043450_zhxk
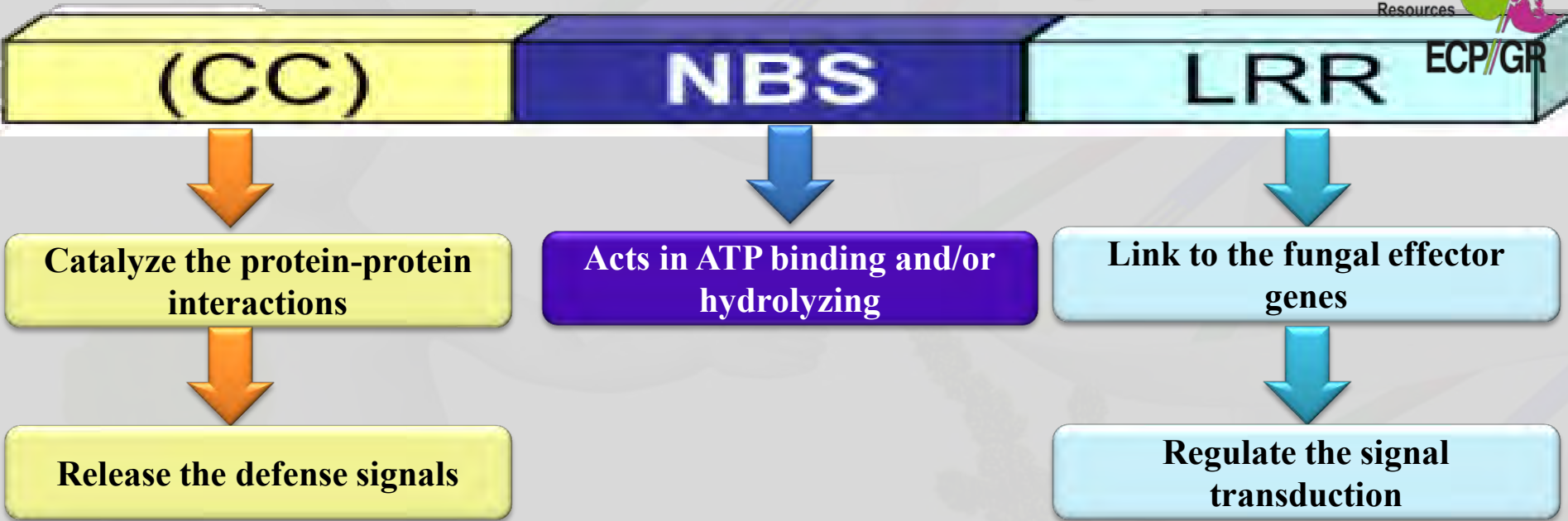
  CC-NBS-LRR

  SNP2

  Bv7_171470_ojty

  NBS-LRR

  NBS-LRR

  Detection of Fusarium effectors

  Hypersensitivity reaction

Functions of resistance-related proteins

(CC)	NBS	LRR

Catalyze the protein-protein interactions	Acts in ATP binding and/or hydrolyzing	Link to the fungal effector genes

Release the defense signals		Regulate the signal transduction

(Martin et al. 2003; Marone et al. 2013)

The allelic variations of both SNPs located in the silent mutation positions and do not change the amino acid sequence

However, it has been found that changes in some synonymous alleles have altered the structure of the protein and, consequently, altered its function (Kimchi-Sarfaty et al. 2007; Komar 2007)

Also changes in synonymous alleles cause a change in the mRNA instability or splicing disruption

Cultivar	Company, Country	Characteristics	SNP1/SNP2	Cultivar	Company, Country	Characteristics	SNP1/SNP2
Aria	SBSI, Iran	Rhizomania/Nemathod	R/R	Loriguet	Florimond Desprez		S/R
BTS213	Beta seed, America		R/hetero	Palma	Maribo Seed	Bolting/ Cercospora	Hetero/hetero
Ekbatan	SBSI, Iran	Rhizoctonia/ Rhizomania	Hetero/hetero	DS4057			Hetero/hetero
Paya	SBSI, Iran	Drought	Hetero/R	Toucan	DEZPREZ, French	Rhizomania/Nemathod	Hetero/hetero
Delta	Sesvanderhave	Rhizomania	R/hetero	Merak	STRUBE, Germany	Rhizomania	S/hetero
Dorothea	Syngenta	Rhizomania (R)/Rhizoctonia (t)	Hetero/hetero	Linda	US Agriseeds		S/hetero
Novodoro	Syngenta, Sweden	Rhizomania/Rhizoctonia	S/hetero	Perfecta	Lion seed		Hetero/hetero
Azare	SESVANDERHAVE	Rhizomania(R)/Rhizoctonia(t)	Hetero/hetero	Rosier	DEZPREZ, French	Rhizomania	S/R
Ukraine	Ukraine	Rhizoctonia	Hetero/hetero	Isabella	KWS, Germany	Rhizomania	S/S
Murialle	DEZPREZ, French	Rhizomania	R/hetero	Baloo	SESVANDERHAVE		Hetero/hetero
Shokofa	SBSI, Iran	Rhizomania/Nemathod	Hetero/R	Ghazira	Khun &co, Netherlands		S/hetero
BTS233	Beta seed, America		Hetero/hetero	Antek	STRUBE, Germany	Rhizomania	Hetero/R
Pars	SBSI, Iran	Rhizomania	Hetero/hetero	Sharif	SBSI, Iran	Bolting	Hetero/R
Flores	DANISCO, Denmark	Rhizomania/Rhizoctonia	S/hetero	Chimene	Florimond Desprez		S/Hetero
Rajah	Sesvanderhave	Bolting	R/hetero	BTS335	Beta seed, America	Rhizomania	S/Hetero

Table 4. Most favorable commercial cultivars in Iran and their resistance characteristics

Sugar beet wild relatives genotyping

Is any allele difference in conserved domains on the location of two SNPs in the wild relatives?
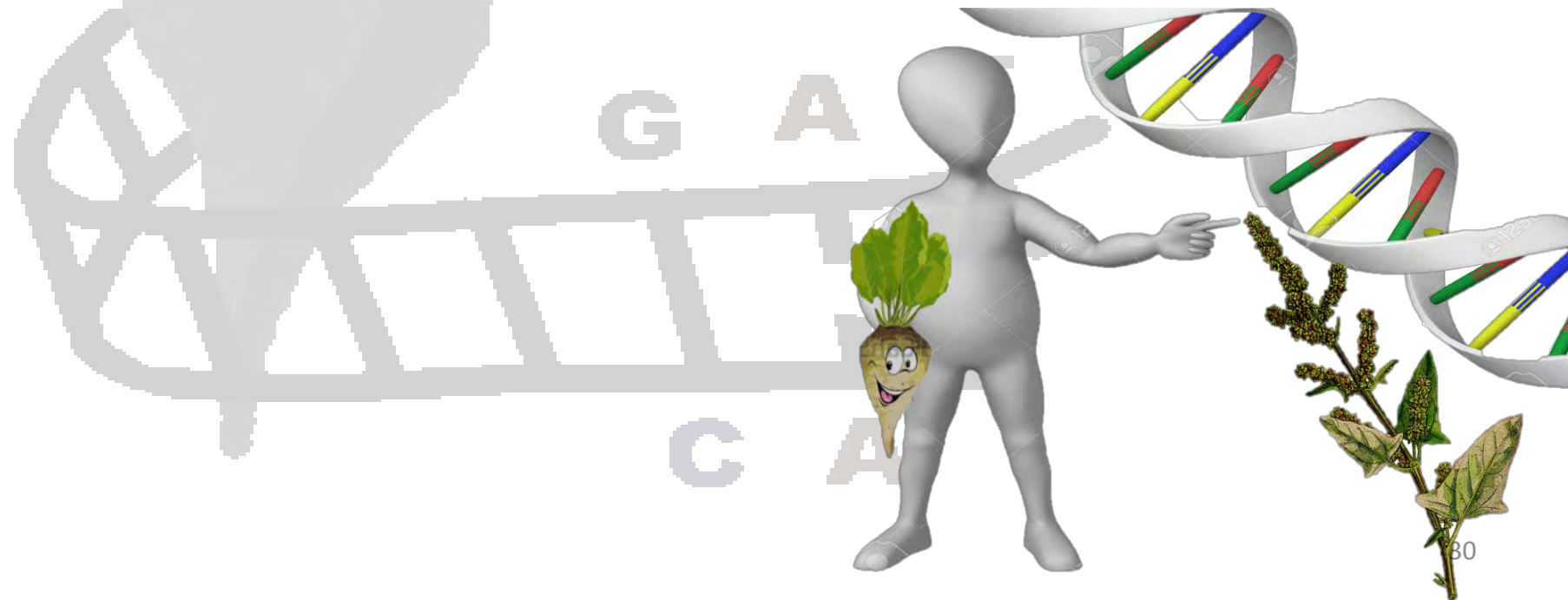
Wild beet genotyping results

1. The association between two SNP mutations, published by De Lucchi et al., 2017, and Fusarium resistance was further validated on wild and cultivated plants using rhAmp technology.

2. Fusarium resistance genes have been inherited from wild type primary ancestors to the cultivated beets

3. There are different evolutions of defense genes in the ancestors of wild beet

5. Geographical difference of the sea beet accessions had a great influence on host-pathogen evolution

6. Our best result thanks to the geographically diverse collection collected by DAFNAE laboratory

Introduction of multi resistance wild beet

- **The *crv* species showed the highest frequency of Fob resistance genotypes**

- **The *crv* species are high tolerance to drought and salinity stress (Stevanato et al. 2012)**

- **There are some rhizomania resistance related SNPs in *Crv* species (Chiodi et al. 2018)**

- **Therefore, the *crv* species is a valuable resistant source against Fusarium, rhizomania, soil salinity and drought conditions with an optimal root system for genetic breeders**

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Conclusions

1. The study introduced Fusarium-resistant commercial cultivars for Iran sugar beet cultivations with a history of severe losses

2. This study is an initial step for future improvement of sugar beet resistance against *F. oxysporum*

3. For the first time, wild beets from Adriatic coast have been screened for resistance to *F. oxysporum*

4. These SNPs have good screening abilities in wild ancestors because of being located in conserved areas of resistance genes

5. These accessions will be further evaluated for their use as parental lines in breeding programs aimed to develop sugar beet varieties with greater adaptability to *Fusarium*

Collaborations

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