Development and validation of molecular markers associated to sweet cherry agronomical traits for breeding programs

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Introduction, context and objectives

In sweet cherry, most agronomic traits are controlled by many genomic regions (QTLs) and are highly dependent of climatic conditions. Moreover, sweet cherry has a long juvenile period (4-6 years). For these reasons, the introduction of a desired character is a long process. Based on several sweet cherry populations evaluated during many years for fruit weight (FW), fruit firmness (FF), fruit cracking (FC) and productivity (Prod), stable QTLs were identified. The aims of this study were to identify SNPs located within the QTL regions using the genome sequence of the 'Regina' cultivar[1] and transcriptomic data[2], and to transform them into KASP (Kompetitive allele specific PCR) markers. These low-cost markers can be easily used for MAB (marker-assisted breeding) in order to allow breeders to increase the efficiency of their programs and to plant only hybrids with favorable allelic combinations for the most critical agronomic traits.

Mat & Methods

An F_1 population obtained from the cross between cultivars 'Regina' and 'Lapins' (n= 118), accessions of Biological Resources Center (BRC; n =165) and in the process of Distinctness, Uniformity and Stability (DUS; n = 62) were evaluated for FW, FF, FC and productivity and genotyped with the KASP markers.
FW average was evaluated on 100 fruits randomly harvested in grams; FF was measured on 10 fruits (2 measures on each cheek) using a Durofel® texture analyzer; FC was evaluated through the visual observation of pistillar end cracking of 50 fruits and Prod was estimated by a score out of nine.

Results

KASP markers have been developed for FW QTL on linkage group (LG) 2 (Fig.1: example for KASP LG2_26_FW), FF QTL on LG4, FC QTL on LG5 and QTL for productivity on LG6. They were validated on the F_1 R×L, BRC and DUS accessions. Significant effects between favourable and unfavourable alleles have been identified for each marker (Tab. 1).

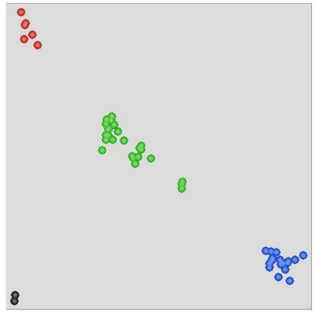

Fig. 1 KASP GL2_26_FW profile for the BRC accessions
Red: CC, green: CT and blue: TT

Tab. 1 KASPs for fruit weight (FW), firmness (FF), cracking (FC) and productivity (Prod) evaluated on F_1 R×L, BRC and DUS accessions.

	R×L				BRC			DUS		
KASP markers	**Geno**	**Ind**	**x̄**	**Gain**	**Ind**	**x̄**	**Gain**	**Ind**	**x̄**	**Gain**
Fruit Weight										
LG2_26_FW	TT	25	8.73	2.08*	49	6.77	2,50*	24	9.90	-
	CC	35	6.65		21	4.27			-	
Fruit Firmness										
LG4_012_FF	AG	63	66.10	2.43*	18	66.9	1.30	7	60.2	-6,70
	GG	55	63.60		147	65.6		38	66.9	
Fruit Cracking										
LG5_4_FC	AA	53	10.30	-8.36*	22	1.36	-0.95		-	
	GA	36	18.70		37	2.32				
LG5_14_FC	TG	64	9.99	-8.99*	27	0.54	-0.85		-	
	GG	53	18.99		135	1.39				
Productivity										
LG6_22_Prod	TA	61	4.72	0.34*	97	4.39	0.06	29	5.86	0,67*
	TT	57	4.38		56	4.33		20	5.19	
LG6_24_Prod	CT	59	4.35	0.48*	79	4.44	0.17	29	6.11	1,13*
	CC	44	3.87		79	4.27		22	4.98	

Comparison of favourable (green) and unfavourable (red) alleles, * : significant p-value < 0,005

Among individuals with favourable alleles for FW, those with favourable alleles for FF and then for FC were selected (Tab.2). These individuals could be used in breeding program as genitors. R×L hybrids and BRC accessions with favourable and unfavourable alleles for the KASP markers are presented in Fig. 2. Among the seven individuals (6 R×L hybrids, 1 BRC accession) with the favourable alleles for the three traits, four of them have the best balance between FW, FF and FC: R×L 10, 44, 53 and 'Uriase-de-Bistrita'. Concerning the three others (R×L 17, 130, 65), their susceptibility to cracking is high, similar to most R×L hybrids that were affected by important rainfall during the evaluated seasons. By crossing R×L 10 (high values for FW and FF) and 'Uriase-de-Bistrita' (good values for FF and FC), hybrids with high values for the three traits can be expected.

Tab. 2 Marked-assisted breeding on F_1 R×L, BRC and DUS accessions.

	Ind	GL2_26_ FW	GL4_12_ FF	GL5_4_ FC	GL5_14_ FC	GL6_22_ Prod	GL6_24_ Prod	FW	FF	FC	Prod
R×L	RxL17	TT	AG	AA	TG	TA	CT	8.7	64.8	17.1	6.0
	RxL44	TT	AG	AA	TG	TA	CT	8.2	68.7	5.5	4.6
	RxL53	TT	AG	AA	TG	TT	CT	8.4	61.1	1.7	5.0
	RxL10	TT	AG	GG	TG	TT	CT	10.1	44.1	12.2	1.8
	RxL130	TT	AG	AA	TG	TA	CT	9.5	53.6	15.9	4.5
	RxL65	TT	AG	GG	TG	TT	CC	10.0	60.2	23.5	6.0
BRC	Uriase-de-Bistrita	TT	AG	GG	TG	TA	CT	7.7	68.1	0.2	2.9
DUS	Fertard	TT	AG	NA	NA	TT	CT	11.4	66.2	NA	4.4
	OBT_INRA_4L15	TT	AG	NA	NA	TT	CC	NA	NA	NA	5.1
	SUNBURST	TT	AG	NA	NA	TT	CT	10.0	49.5	NA	6.3
							Means	9.3	59.6	10.9	4.7
							Max	11.4	68.7	23.5	6.3
							Min	7.7	44.1	0.2	1.8

Green : favourable allele and Red : unfavourable allele, NA : missing data

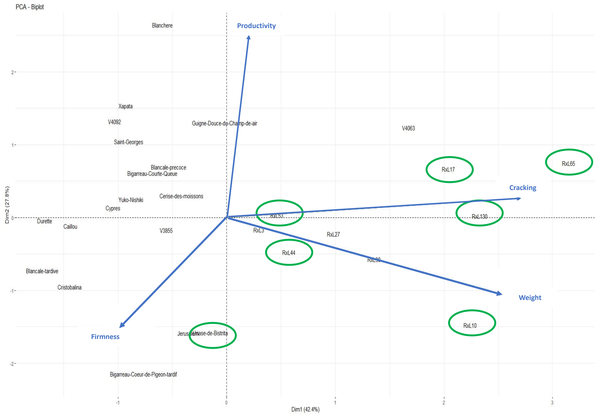

Fig. 2 PCA of favourable and unfavourable individuals for the fruit weight, fruit firmness, fruit cracking and productivity on F_1 R×L and BRC accessions.
Favourable in green and unfavourable in black

Conclusions and perspectives

KASP markers were identified and validated for FW, FF, FC and Prod. Good genitors for all traits were not yet identified. However, by crossing individuals with favourable complementary alleles, hybrids with good fruit quality value could be used as genitors in breeding programs.

References: [1]Le Dantec et al (2020); [2]Vimont et al (2018)
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