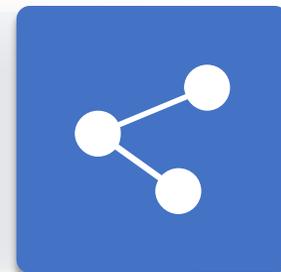




Genetic Mapping and a New PCR-based Marker Linked to Dwarfing Gene in Oat (*Avena sativa* L.)

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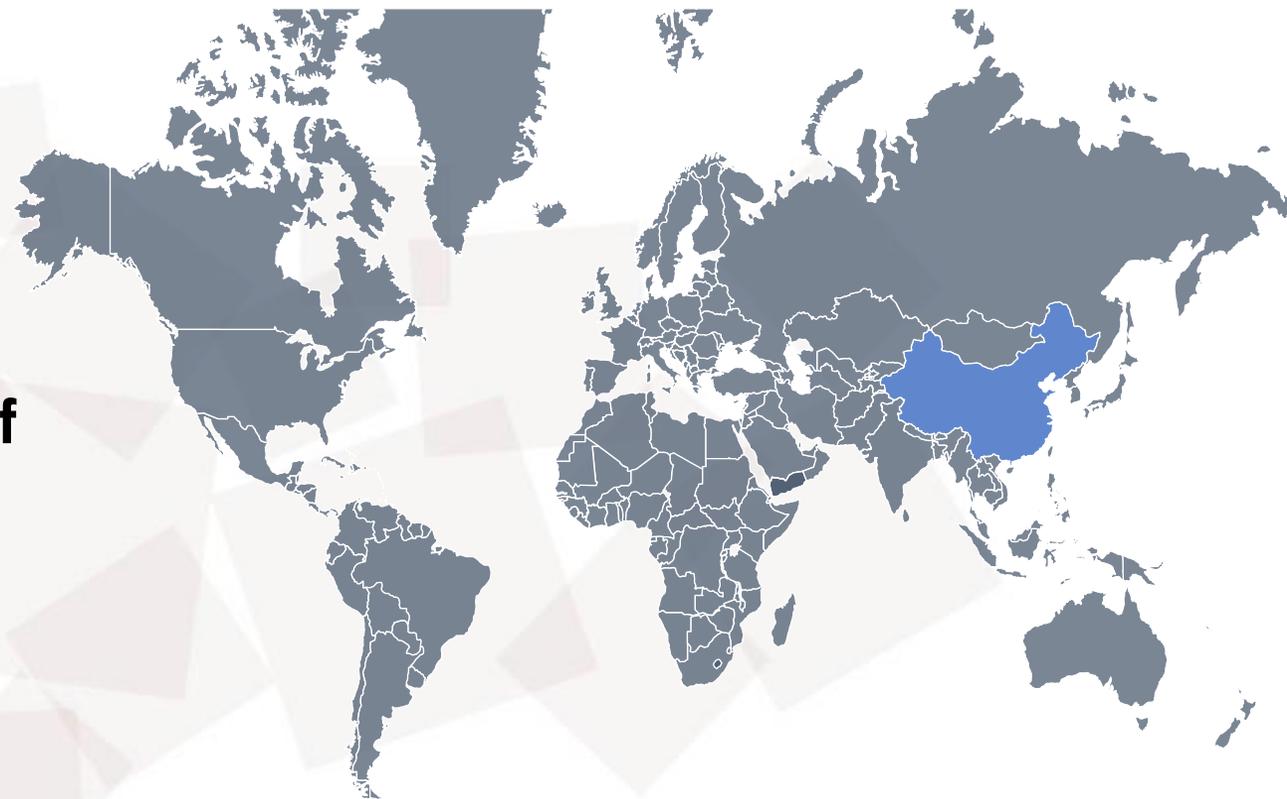


1

RESEARCH BACKGROUNDS



- Oat provides high levels of nutrient components (**protein**, **unsaturated lipids**, **vitamins** and **dietary fibers**) and is widely used in human and animal diets.
- In China, one of the major problems of the oat crop is **lodging**, which may cause severe losses in grain yield and quality.
- In the production of oat, grain yield as well as standability and harvestability may be affected by plant height.



Resistance to lodging can be improved by reducing plant height



● The use of major genes to reduce plant height in crops such as **wheat** *Rht1*、*Rht2*、*Rht8*、*Rht12*、*Rht13* (*Triticum aestivum* L.) (Rebetzka et al. 2011; Cui et al. 2011; Zanke et al. 2014.), **rice** *IR8* (*Oryza sativa* L.) (Kovi et al. 2011; Liu et al. 2013) has resulted in the development of cultivars with increased grain yield (Gale and Youssefian 1985, Chandler and Harding 2013).

● As a consequence of the impact of these genes in wheat and rice, extensive searches for mutants with reduced plant height have been conducted in other cereal species (Kumar et al. 2013; Xing et al. 2015).

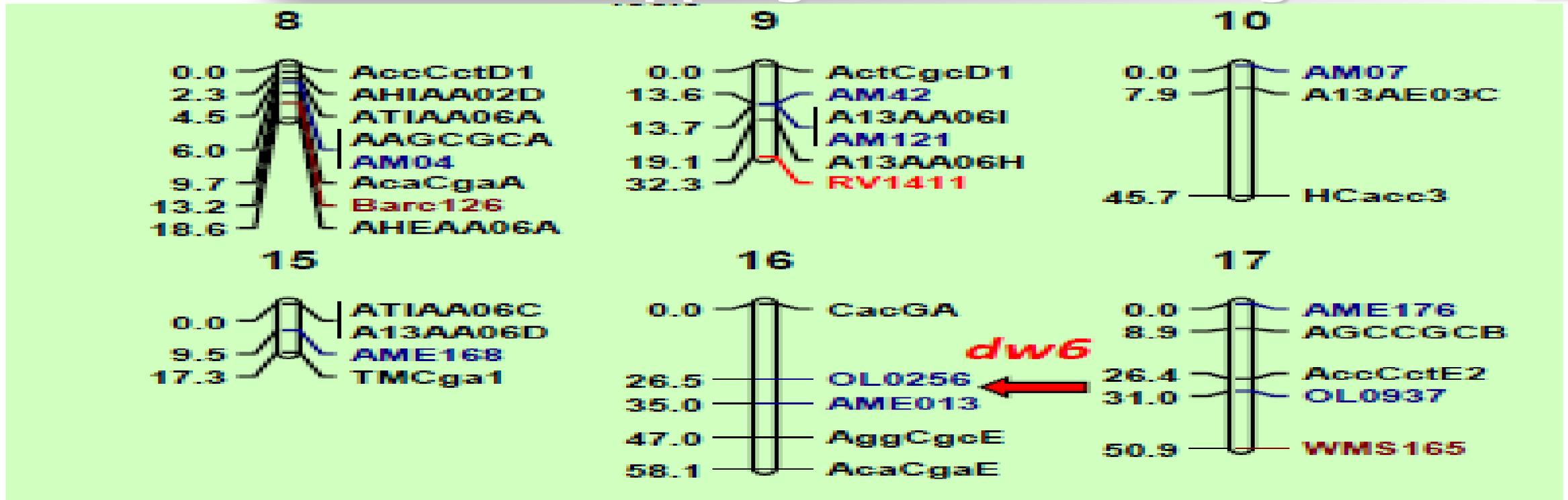
Maize: *D8*、*D9*、*Br2* ; **Barely:** *sdw1*、*Sdw3*、*Uzu et al.*



Oats with short stature were first reported by Warburton (Warburton 1919), subsequent work to characterize dwarf oat was performed (Derick 1930; Litzenberger 1949). It was found that dwarfing genes could simultaneously increased yield and reduced stature as well as lodging susceptibility.

Since then, attempts have been made to reduce the plant height of oat by major dwarfing genes, and eight dwarfing genes (*Dw1* to *Dw8*) have been described

Name	Characteristic	Reference
<i>Dw1</i>	recessive	Marshall and Murphy 1981
<i>Dw2</i>	small effect	Federizzi and Qualset 1989
<i>Dw3</i>	small effect	Tanhuanpää et al. 2006
<i>Dw4</i>	similar plant type	Derick 1930
<i>Dw5</i>	recessive	Nishiyama 1957
<i>Dw6</i>	dominant	Brown et al. 1980
<i>Dw7</i>	partially dominant	Marshall and Murphy 1981
<i>Dw8</i>	extrem dwarf	Milach et al. 1997



AME013 in an F2 mapping population of the cross between Buffalo and Tardis, **and the distance of the two SSR markers was 8.5 cM.**

- Molnar et al.(2012) located the Dw6 locus to a small chromosomal region on K×O linkage group (LG)KO33, near or at a putative PFLP locus *aco245z*. **That gives us an opportunity of designing highly precise diagnostic markers for MAS for Dw6.**



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FOUNDATION OF TOPIC



The **dwarfing genes** both increased yield and reduced stature, the latter also increasing lodging resistance.

Although the genetic mapping of oat **dwarfing genes** has been reported, and there is considerable interest in deploying dwarfing genes in oat breeding programs, molecular markers linked tightly to dwarfing genes are not readily available. This situation would be improved by:

- 1 development of simple PCR-based assays that are tightly linked to dwarfing genes
- 2 through better characterization of map positions, which would provide access to high-throughput marker assays

To identify PCR-based markers associated with the **dwarfing gene** in oat variety WAOAT2132 using bulked-segregant analysis (BSA), and to localize these to a high-density reference map.



3

PLANT MATERIALS



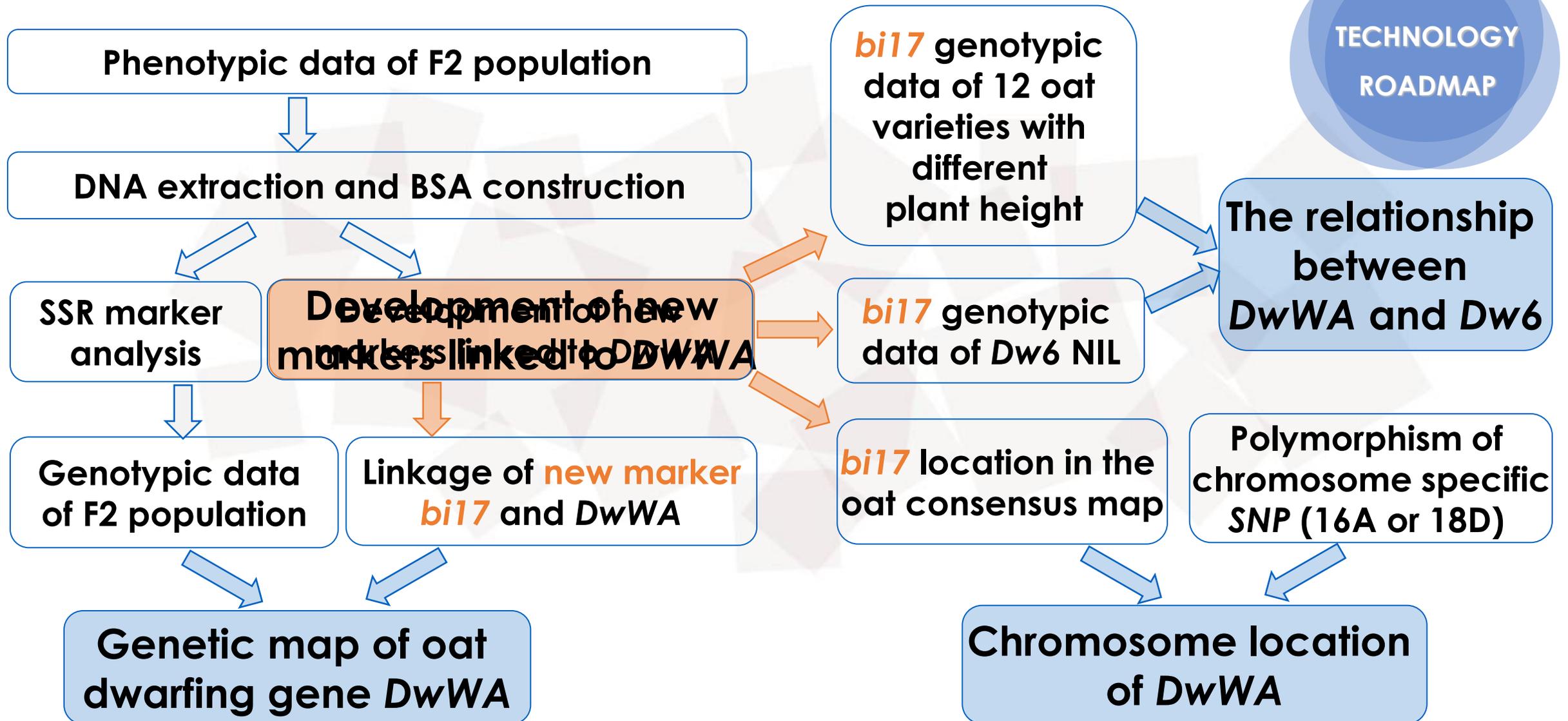
- 306 F2 individuals of WAOAT2132×Caracas mapping population
- 39 progenies derived from Dal × Exeter population
- 12 oat varieties with different plant height including Kanota, Ogle, Terra, Marion, Dal, Exeter, OT207 and Potoroo et al.
- 14 individuals of the near isogenic line (NIL) which was generated for *Dw6*

*The oat varieties, the progenies of D×E population and the NIL individuals were provided by Dr. Nicholas A. Tinker, Eastern Cereal and Oilseed Research Centre, Agricultural & Agri-Food Canada (AAFC).



4

TECHNOLOGY ROADMAP



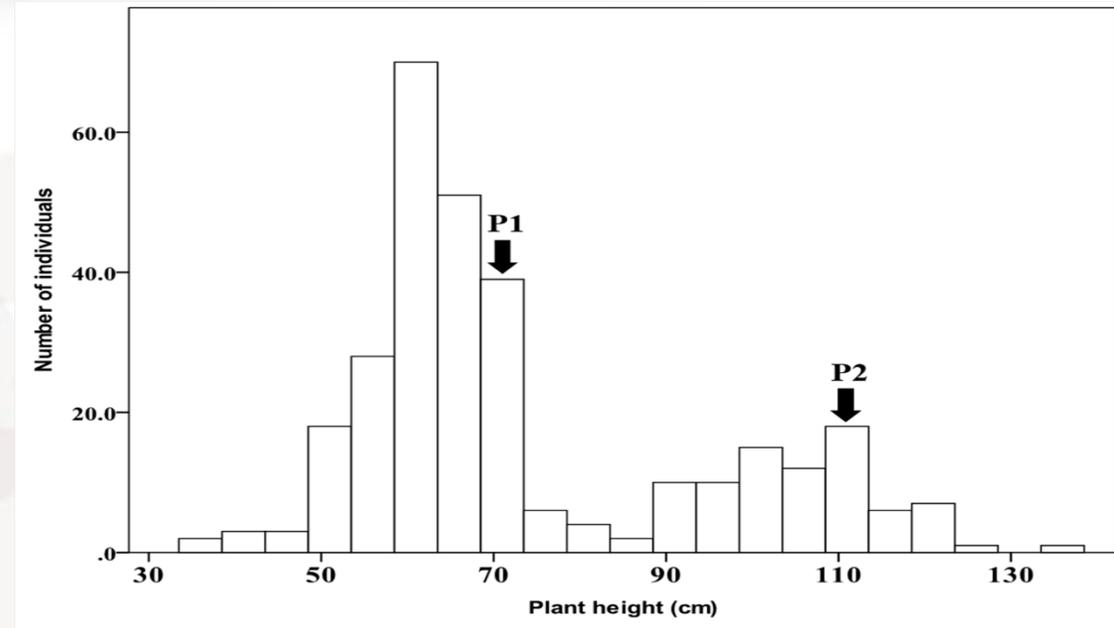


5

RESULT AND ANALYSIS



The average height of
WAOAT2132 : 70 cm
Caracas: 110 cm.



Plant heights in the F2 population varied from 36 to 135 cm.

- The distribution of heights indicates a monogenic dominant inheritance of the dwarfing gene.

- We were able to classify the F2 individuals into homozygotes (non-segregating progenies) and heterozygotes (segregating progenies) by measuring the heights in F2:3 progenies.



- The total number of primer pairs derived from oat and barley was **102**, including AME SSRs, AM SSRs, MAMA SSRs, and OL0256.
- Of them, the four primer pairs, **AME117**, **AME013**, **AME055** and **OL0256** were **polymorphic** between both of the contrasting bulks and parental lines.

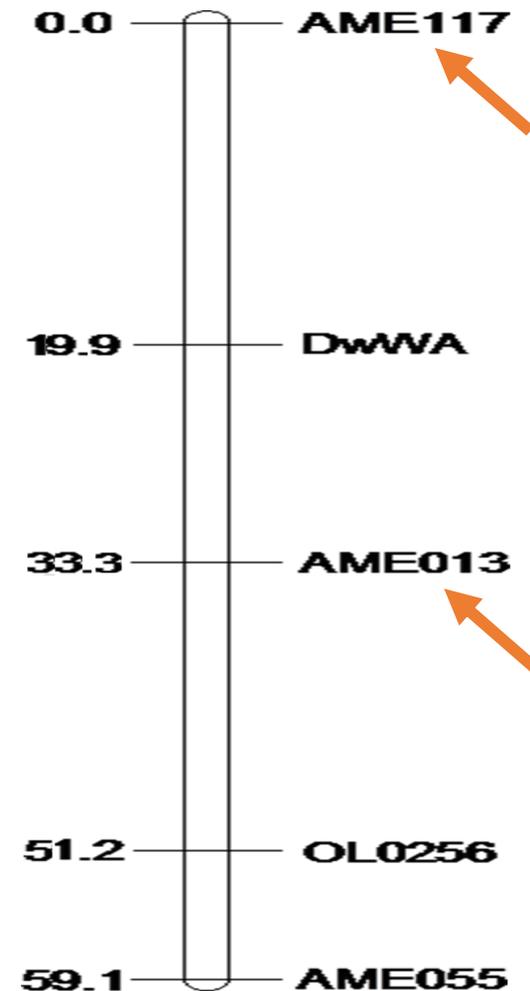
Primers of the SSR markers linked to *DwWA*

Marker	Forward primer	Reverse primer
<i>AME013</i>	GGCATGAGAGTTTTATGAACC	ACGGAACTTCAACACTTTGG
<i>AME055</i>	CGGAGGTGCAAACCCTAGTA	TTCGACCATGGGAATCTTG
<i>AME117</i>	GCAACTTCATGTCCAGTACTC	GTTTCGCAAGGCAAGGAAC
<i>OL0256</i>	GTGTGCATCCCTCATCAGTC	ACCTCCATCTCGACCTCAACT



These four SSR markers were completely associated with oat dwarfing gene *DwWA*, and associated with each other as well.

Initial genetic mapping showed that oat dwarfing gene *DwWA* was bracketed by the two SSR marker loci *AME013* and *AME117* at map distances of 13.4 and 19.9 cM, respectively

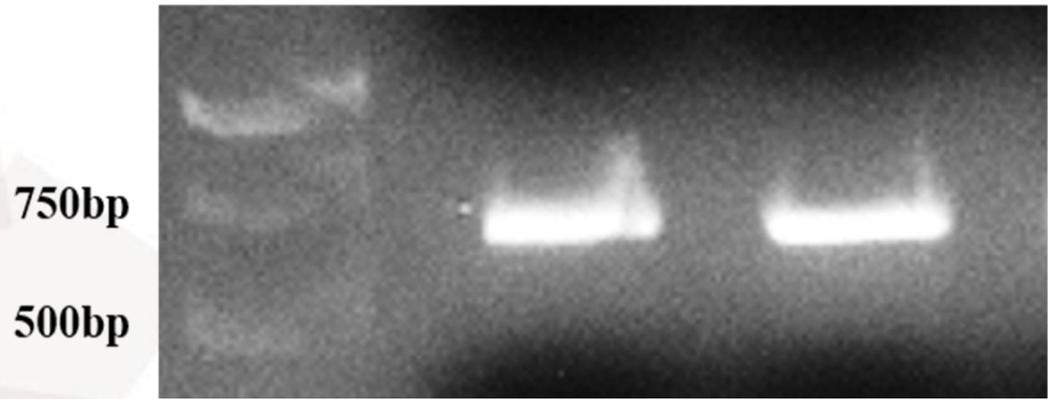


Initial genetic map of oat dwarfing gene *DwWA* based on SSR polymorphisms from 306 F₂ families of WAOAT2132×Caracas population. Genetic distances in cM are given on the left and marker loci are named on the right side of the map.



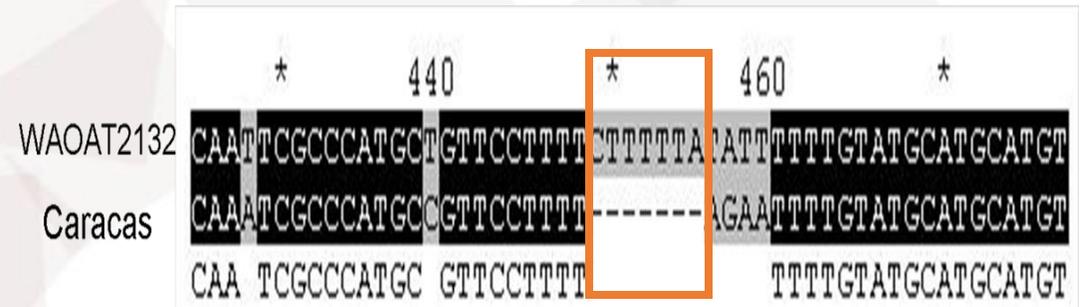
To search for new markers closer to *DwWA* than the SSR markers, the primers were designed based on *aco245* to amplify the sequences in WAOAT2132 and Caracas and the PCR products was about 720 bp

WAOAT2132 Caracas



Products of primer 17F/R in WAOAT2132 and Caracas

The sequence of 'WAOAT 2132' differs from that of 'Caracas' which has 7 nucleotide lost and the difference of the loci which may link to oat dwarfing gene



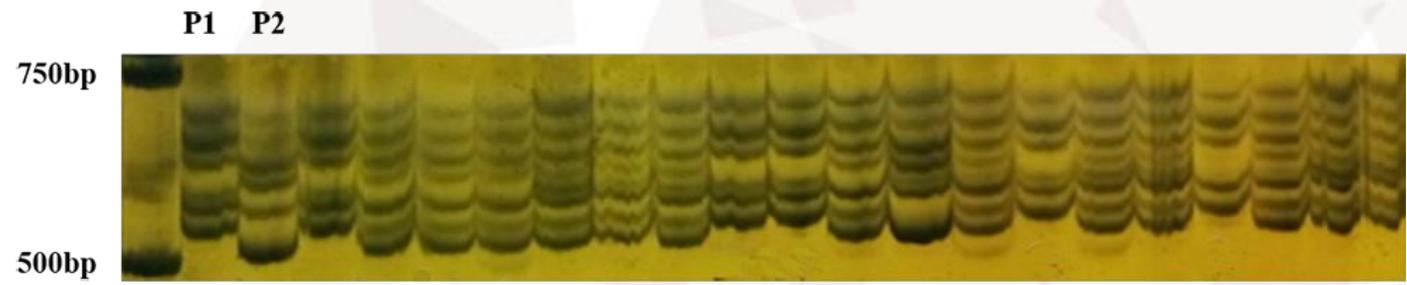
Sequence difference between WAOAT2132 and Caracas which was used to design *bi17* primers

Therefore, a new PCR-based codominant marker named *bi17* was developed

Linkage of *bi17* and *DwWA*

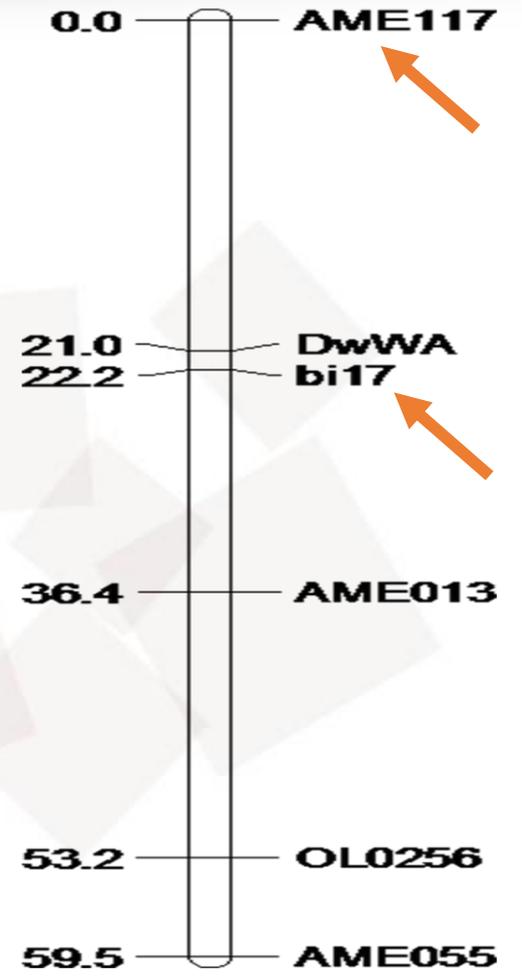


- The new marker *bi17* was closely linked to the oat dwarfing gene *DwWA*. Only 8 individuals were wrongly classified or recombination occurred in the whole population, the effective error rate (Penner et al. 1995) for the *bi17* is only 2.6% (8/306).



Example of marker production profiles of the primer pairs *bi17* in parents and F2 families from WAOAT2132×Caracas. P1: WAOAT2132, P2: Caracas.

Based on the method of maximum likelihood linkage analysis with the phenotype and genotype data of the F2 plants and F2:3 families, *DwWA* was flanked by *AME117* and *bi17* with genetic distances of 21 and 1.2 cM, respectively.



The LOD score between *bi17* and *DwWA* was 121.18 and 23.15 between *AME117* and *DwWA*.

Chromosome location of DwWA



The SNP marker *GMI_ES_LB_11151* derived from an EST sequence which has a good match to **aco245** was mapped on chromosome 16A, and is highly homeologous to chromosome 18D, but **there is an SNP on this locus that is different between 16A and 18D**. To test this SNP, we design a pair of primer *KZ11* to amplify WAOAT2132, Caracas and some of progenies of our mapping population.

The SNP site is **G** in WAOAT2132, Caracas and all of the progenies that we amplified.

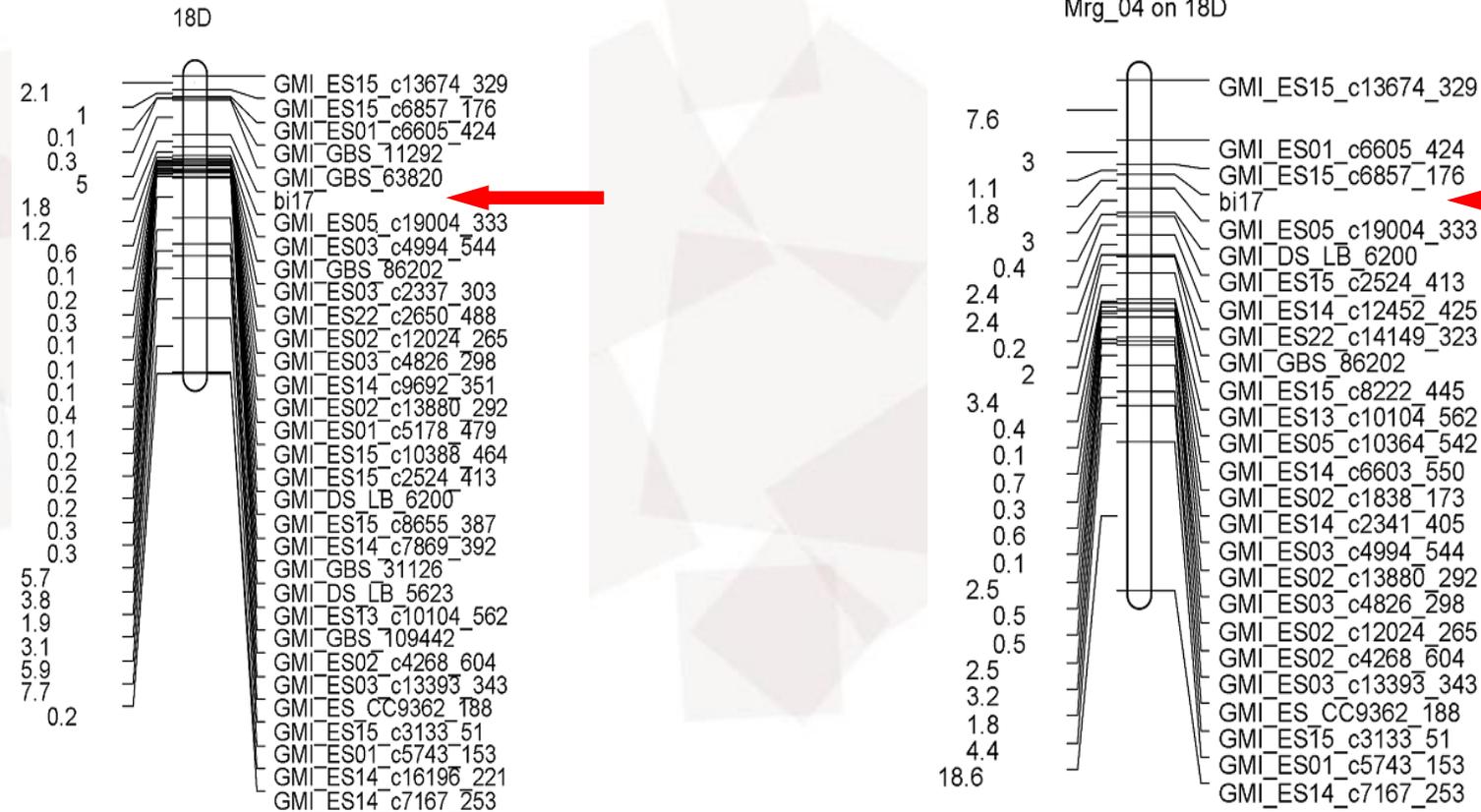
ACO245_vacuolar_H+-ATPase_subunit_H_mRNA	TTGATTATTTGTCCACTACAAGGGTTATG	CCAAGACTTGTAGAAGTTGTC
usda_ars_84035.7_SunII_c708	TTGATTATTTGTCCACTACAAGGGTTATG	CCAAGACTTGTAGAAGTTGTC
usda_ars_84035.11_Tardis_c5762	TTGATTATTTGTCCACTACAAGGGTTATG	CCAAGACTTGTAGAAGTTGTC
dwarf_individual_SNP	TTGATTATTTGTCCACTACAAGGGTTATG	CCAAGACTTGTAGAAGTTGTC
tall_individual_SNP	TTGATTATTTGTCCACTACAAGGGTTATG	CCAAGACTTGTAGAAGTTGTC
WAOAT2132_SNP2	TTGATTATTTGTCCACTACAAGGGTTATG	CCAAGACTTGTAGAAGTTGTC
Caracas_SNP2	TTGATTATTTGTCCACTACAAGGGTTATG	CCAAGACTTGTAGAAGTTGTC
Consensus	ttgattatTTGTCCACTACAAGGGTTATG	ccaagacttgtagaagttgtc

↑
SNP

■ The new maker which closely linked to the oat dwarfing gene *DwWA* should be on 18D.



Dal, Exeter and 39 progenies derived from Dal × Exeter population were used to **combine the marker *bi17* in Dal × Exeter consensus map.**



It indicated that the marker ***bi17*** was linked to the SNP markers which were chose from oat chromosome 18D

The relationship between DwWA and Dw6

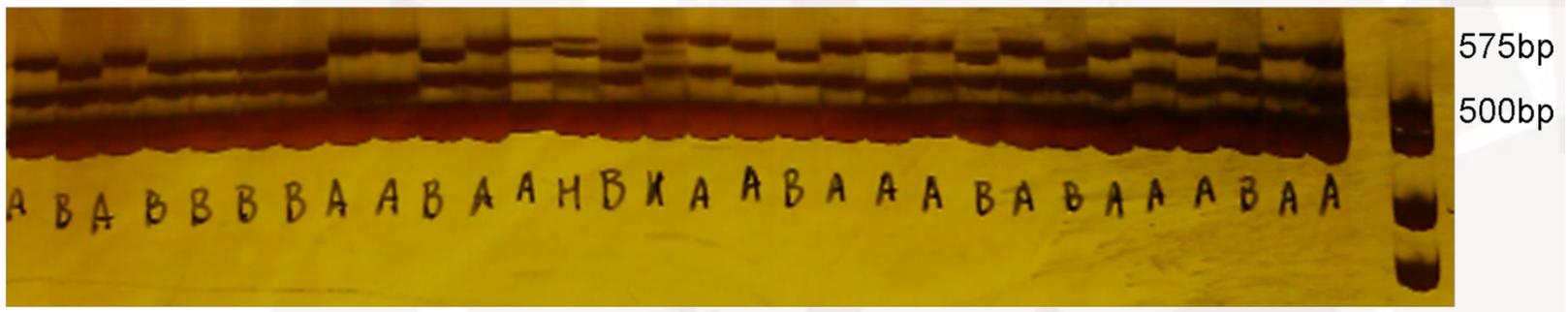


● marker **bi17** was used to amplify some materials, including the parents of mapping population, the dwarf and tall individual DNA pool, some individuals of the NIL generated for *Dw6* and some other non-related lines

The materials used for Allelism test between *DwWA* and *Dw6*.

No.	Material name	Phenotype	Genotype
1	WAOAT2132	Dwarf	A
2	Caracas	Tall	B
3	Dwarf DNA pool	Dwarf	A
4	Tall DNA pool	Tall	B
5	Kanota	Tall	B
6	Ogle	Dwarf	B
7	Terra	Tall	B
8	Marion	Tall	A
9	Dal	Tall	A
10	Exeter	Tall	B
11	OT207	Dwarf	A
12	OT257	Dwarf	A
13	OT526	Tall	AB
14	Jasper	Tall	B
15	N326-7	Tall	AB
16	517-01	Tall	A
17	517-01	Dwarf	A
18	521-02	Tall	B
19	521-02	Dwarf	A
20	521-03	Tall	A
21	521-03	Dwarf	A
22	524-02	Tall	B
23	524-02	Dwarf	A
24	525-01	Tall	B
25	525-01	Dwarf	A
26	525-02	Tall	C
27	525-02	Dwarf	A
28	525-03	Tall	B
29	525-03	Dwarf	A
30	Potoroo	Dwarf	B

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 M



● The same fragment was amplified in WAOAT2132, the dwarf individual DNA pool, OT207, OT257, Potoroo and all of the dwarf individuals of the *Dw6* NIL;

● Caracas, the tall individual DNA pool, Jasper, the tall NIL individuals 521-02, 524-02, 525-01, 525-03 has another kind of fragment; N326-7, the tall NIL individuals 517-01, 521-03, 525-02 also has the dwarf stalk kind of fragment

■ **DwWA is the same as Dw6**



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SUMMARY

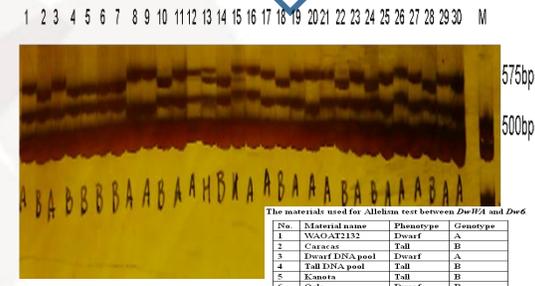
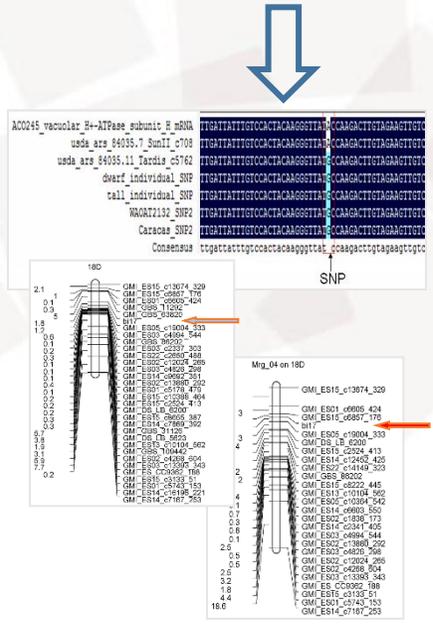
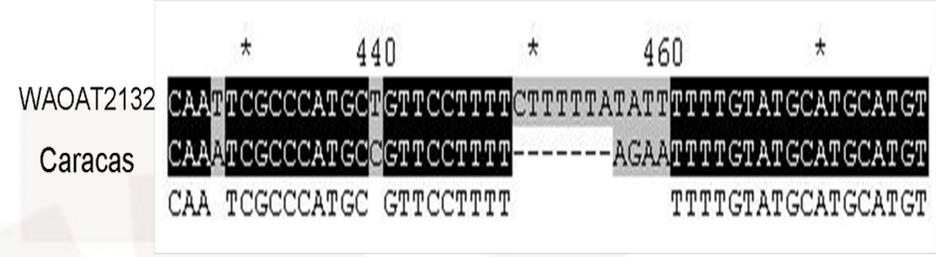
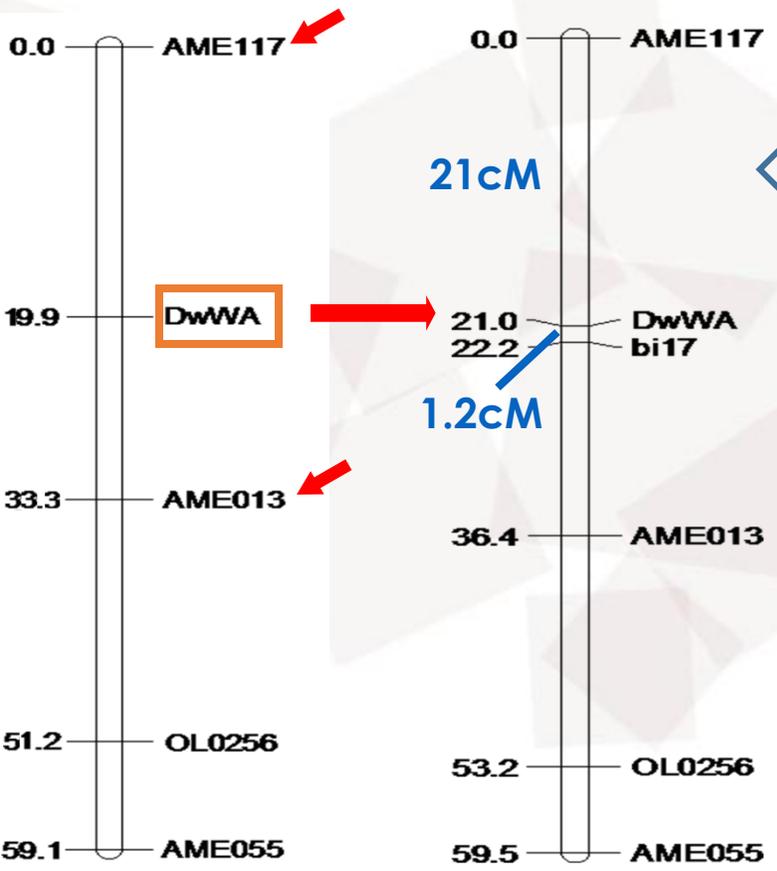
6 SUMMARY



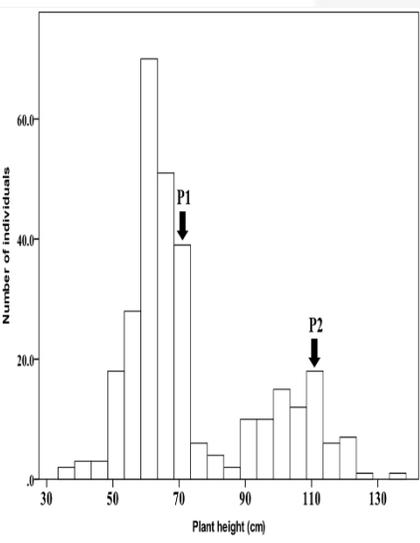
Phenotypic data of F2 population

WAOAT2132/Caracas F2 and RILs

Development of new marker (*bi17*) linked to *DwWA*



No.	Material name	Phenotype	Genotype
1	WAOAT2132	Dwarf	A
2	Caracas	Tall	B
3	Dwarf DNA pool	Dwarf	A
4	Tall DNA pool	Tall	B
5	Kanara	Tall	B
6	Osde	Dwarf	B
7	Terra	Tall	B
8	Talman	Tall	A
9	Dal	A	
10	Exeter	Tall	B
11	OT200*	Dwarf	A
12	OT25*	Dwarf	A
13	OT226*	Tall	AB
14	Esger	Tall	B
15	N326.7	Tall	AB
16	511-01	Tall	A
17	511-01	Dwarf	A
18	521-02	Tall	B
19	521-02	Dwarf	A
20	521-03	Tall	A
21	521-03	Dwarf	A
22	524-02	Tall	B
23	524-02	Dwarf	A
24	525-01	Tall	B
25	525-01	Dwarf	A
26	525-02	Tall	C
27	525-02	Dwarf	A
28	525-03	Tall	B
29	525-03	Dwarf	A
30	Patorno	Dwarf	B



Genetic map of oat dwarfing gene *DwWA*

Chromosome location of *DwWA* (18D)

The relationship between *DwWA* and *Dw6*

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