



Department of Biotechnology
Govt. of India



“Rapid development and characterization of chromosome specific translocation line of *Thinopyrum elongatum* with improved dough strength”

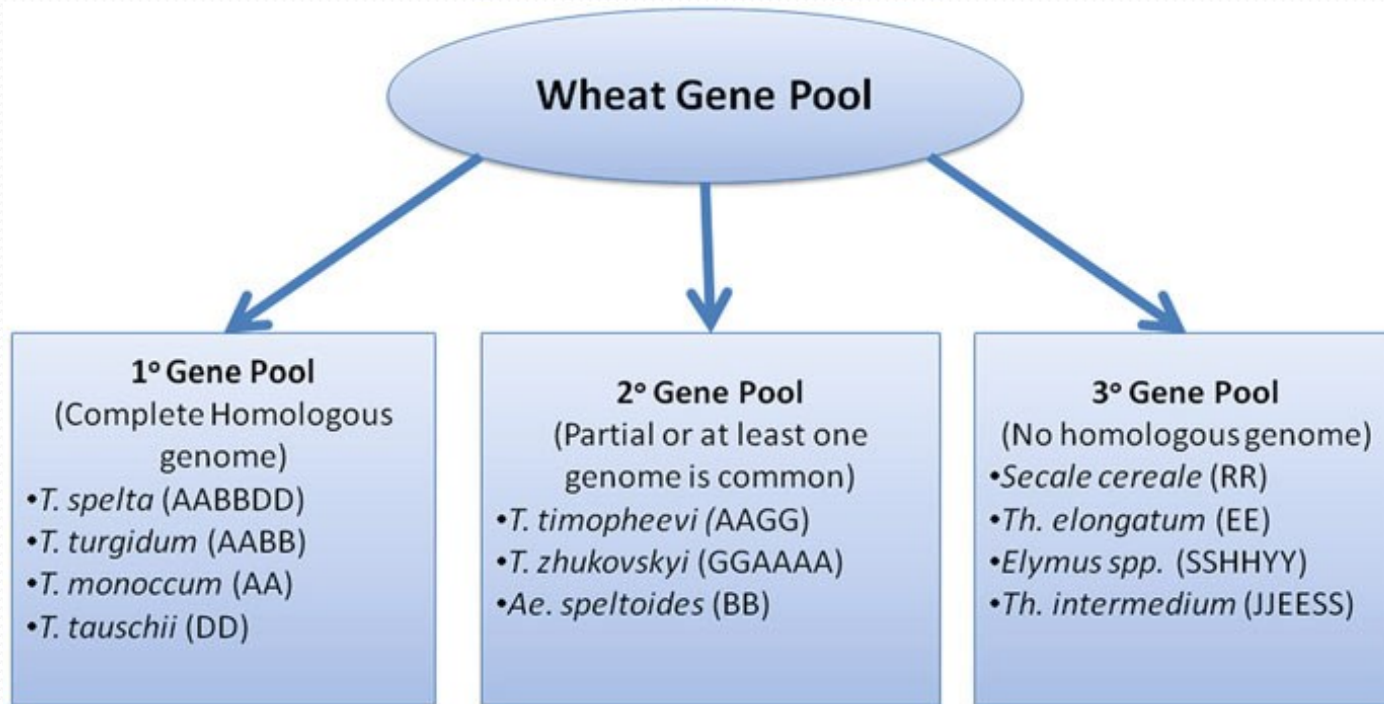
Dr. Monika Garg

Scientist D

NABI, Mohali

Introduction

Under utilized gene pool – a potential gold mine for genetic variability



γ, ω-gliadin

LMW glutenin



HMW glutenin



1A

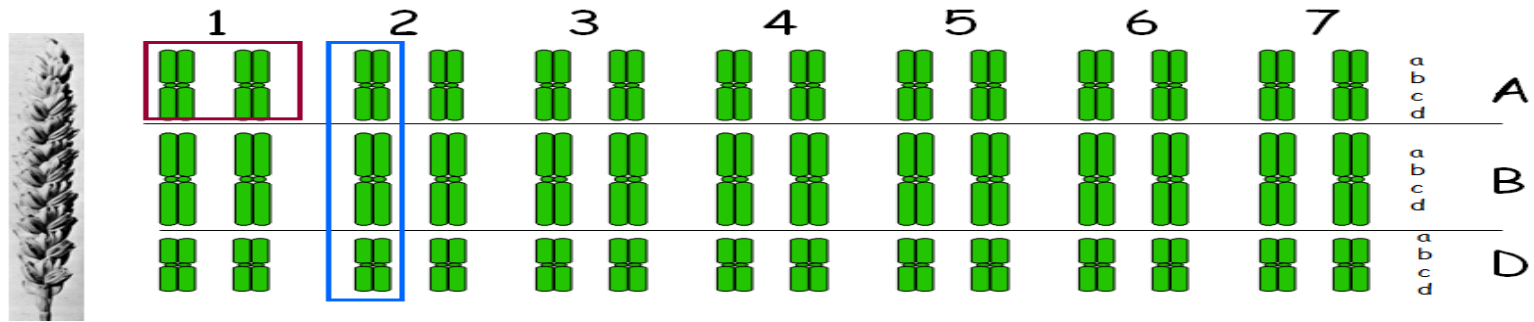


1B



1D

- Major seed storage proteins of wheat –glutenins and gliadins that are responsible for bread making quality
- Our knowledge on orthologous subunits and their effect on end product quality in wheat related species is still limited

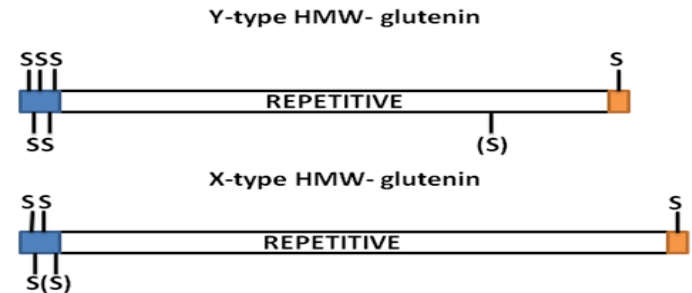
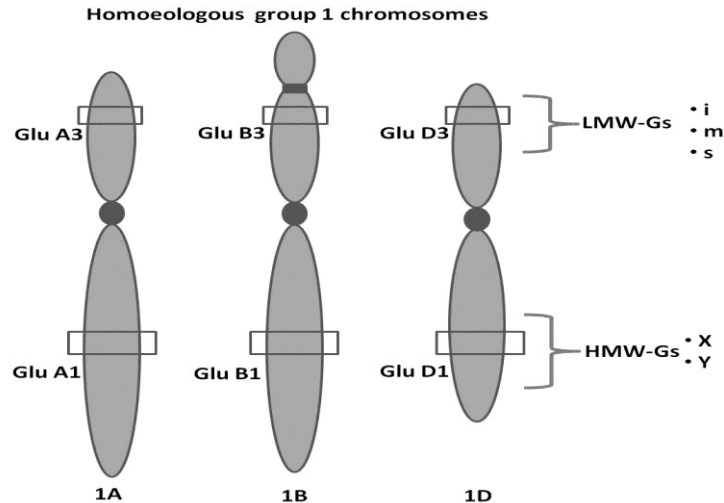


homologues

homoeologues

Diploid - Homologues distinguished from non-homologous chromosomes

Wheat - Homologues, homoeologues and non-homologous chromosomes



Implications of different *Glu1* and *Glu 3* allelic combinations

- Individual allelic effects and interactions of glutenins on processing quality are important
- *Glu D1d*, *GluB1a1*, *Glu A3d* and *Glu B3g* allele having most positive effect
- *GluA1c*, *GluB1a*, *GluA3e* and *GluB3j* have least positive effect on bread making quality

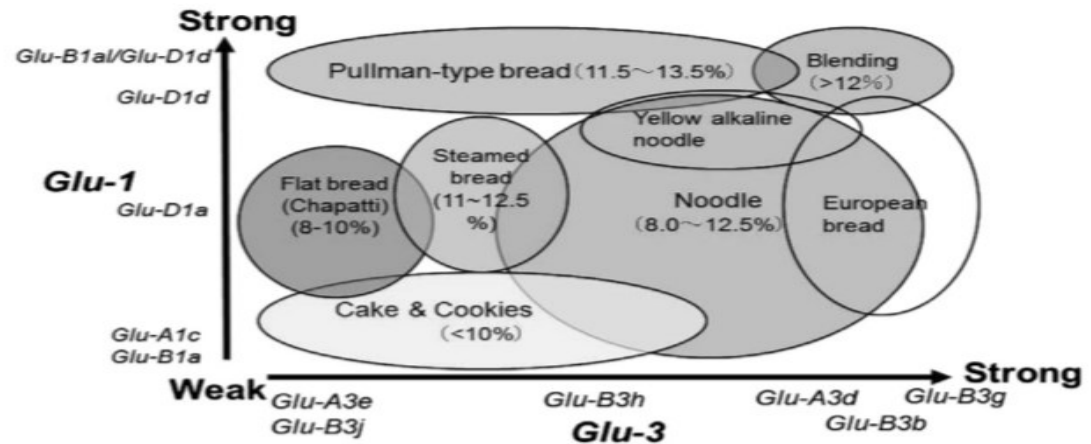


Fig. 2. Breeding implications of glutenin alleles for specific wheat-based products; Y-AXIS represents *Glu-1* alleles and X-AXIS represents *Glu-3* allele effects on dough strength at different grain protein levels (modified from Ikeda and Takata 2013).

- Indian cultivars : poor in bread making quality in spite of GluD1d and GluB1i and GluA1a
- Allelic variations in *Glu-1* loci : highly sought after in quality wheat breeding practice



- Wheat wild species : rich source HMW-GS variations
- The allelic variation of HMW-GS in wheat wild species can be exploited for quality traits

Agropyron elongatum as potential source for end product quality improvement



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Agropyron elongatum HMW-glutenins have a potential to improve wheat end-product quality through targeted chromosome introgression

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Agropyron elongatum

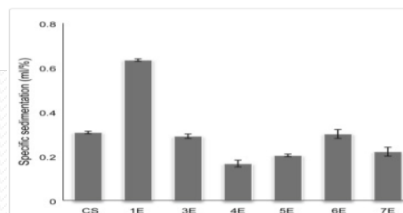


Fig. 2. Comparison of specific sedimentation of DAL1E with other addition lines and Chinese Spring (CS). DAL1E showed a significant increase in specific sedimentation compared to CS and the remaining addition lines.

Dough properties of the homoeologous-group 1 addition line of *Ag. elongatum* (DAL1E) compared with recipient Chinese Spring and the disomic substitution line (DSL1E(1D)).

Line	Protein content (%)	Specific sedimentation	Mixograph (10 g)			SE-HPLC analysis	
			MPT (min)	PH (%)	PBD (%)	UPP (%)	UPP/TPP (%)
CS	10.8 ^a	0.29 ^a	1.3 ^a	45.0 ^a	18.7 ^a	10.1 ^a	37.1 ^a
DAL1E	14.2 ^b	0.63 ^b	2.2 ^b	56.0 ^b	22.0 ^b	13.5 ^b	48.0 ^b
DSL1E (1D)	12.7 ^a	0.19 ^c	0.3 ^c	41.0 ^c	15.7 ^c	7.0 ^c	31.1 ^c

MPT = Mixing peak time, PH = Peak height, PBD = Peak bandwidth. Means followed by the same letter within a column are not significantly different at 5% as determined by the least significant difference.



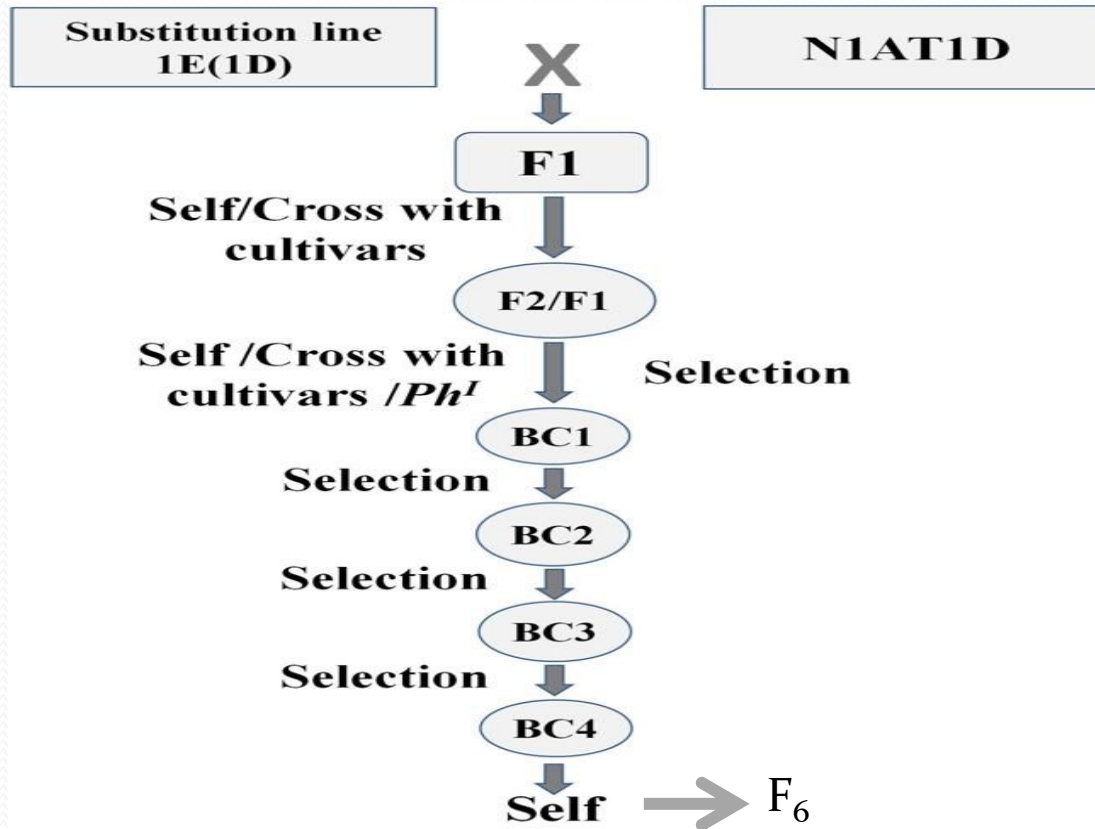
Objectives

- Development and characterization of translocation lines by combination of biochemical, molecular cytogenetic and PCR based molecular markers
- Study of effect of translocations on dough elasticity, extensibility and bread making quality

Plant materials

- Disomic addition of *Th. elongatum* chromosome 1E (DAL-1E) and disomic substitution lines of *Th. elongatum* chromosome 1E with chromosome 1D of wheat both in the background of Chinese Spring (CS) cultivar {DSL-1E(1D)}
- Genetic stocks nulli for chromosome 1A and tetra for chromosome 1D (N1AT1D), N1AT1B, N1BT1A, N1BT1D, and N1DT1B in the background of CS
- Cultivars: AcDomain, Norin 61 (N61), PBW343, Cham 6

Creation of translocation line

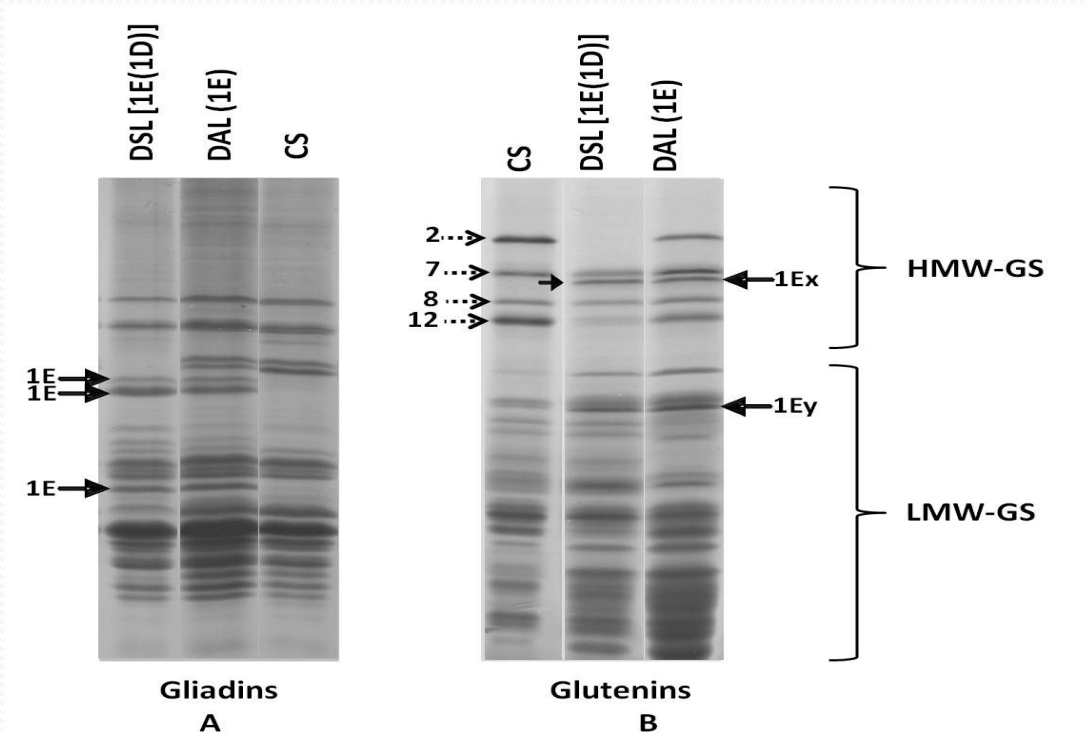


Screening of translocation line

- Identification of *Th. elongatum* specific glutenin and gliadins markers
- Identification of CS (Chinese spring chromosome 1A) specific glutenins and gliadins markers
- Screening of endosperm halves of seeds for presence of *Th. elongatum* specific glutenin (1EL) and absence of *Th. elongatum* specific gliadins (1Es)
- Genomic in situ hybridization (GISH) for the root tips of embryo halves of planted seeds

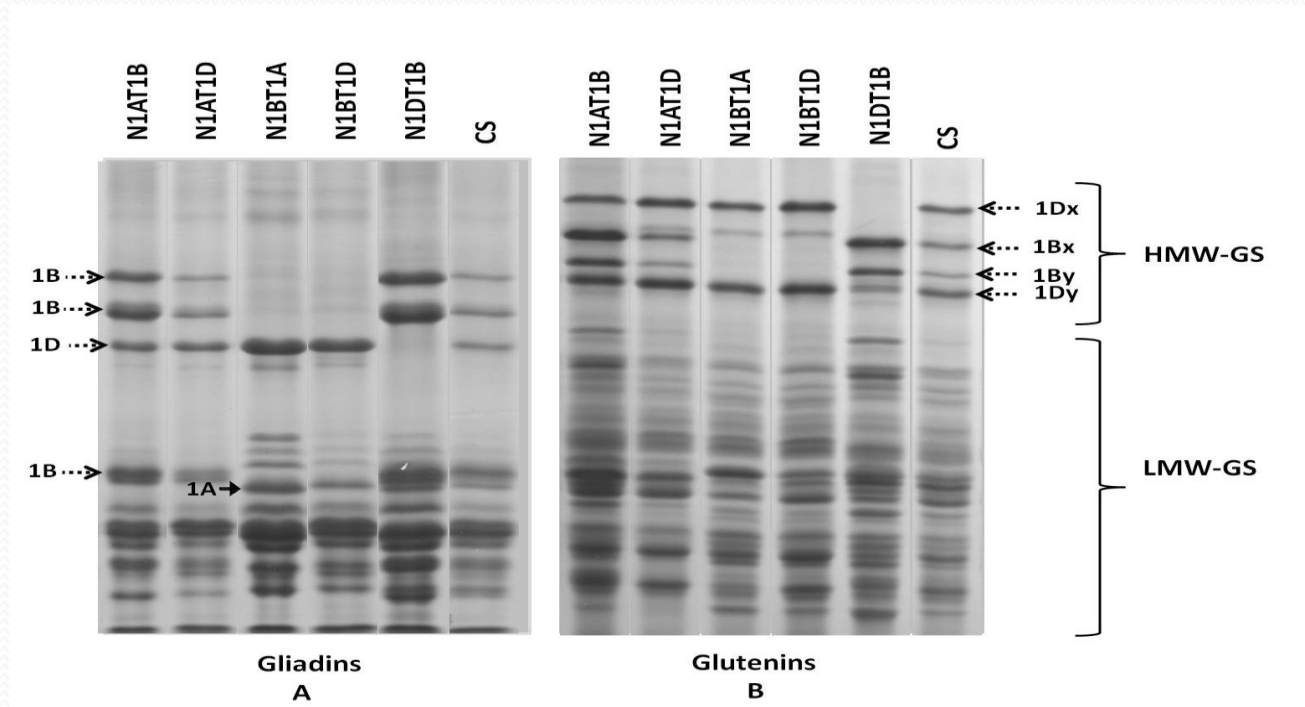
Seed storage protein electrophoretic profiles for identification of *Th. elongatum* specific protein markers.

- Identified two *Th. elongatum* specific glutenins (1Ex, 1Ey) and
- Three *Th. elongatum* specific gliadins

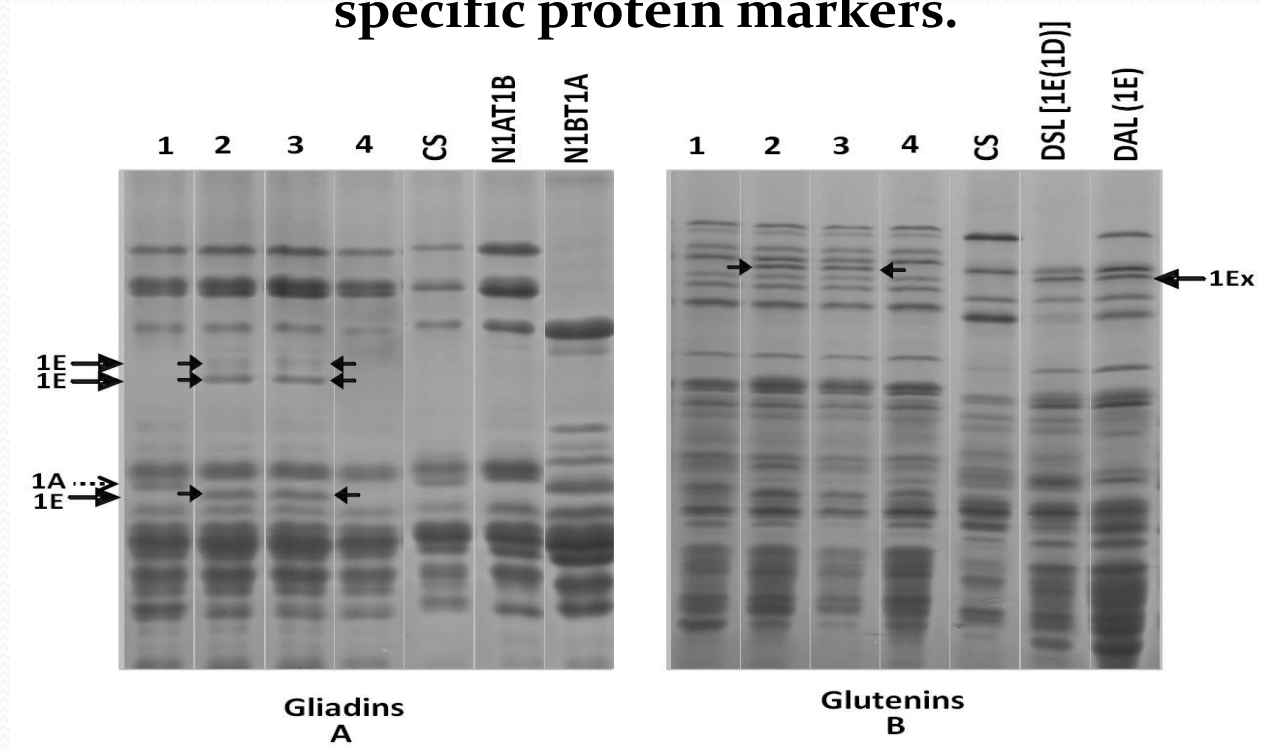


Seed storage protein electrophoretic profiles for identification of Chinese spring (CS) specific protein markers.

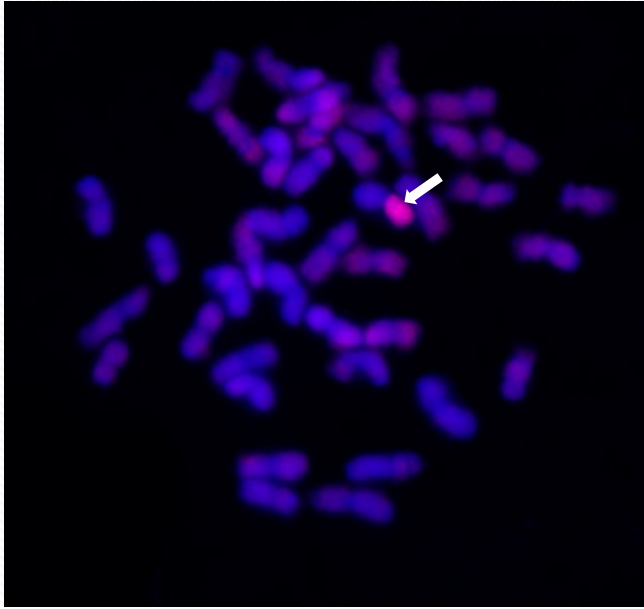
- Identified chromosome 1As specific gliadins for CS
- Absence of Chromosome 1AL specific glutenins in CS



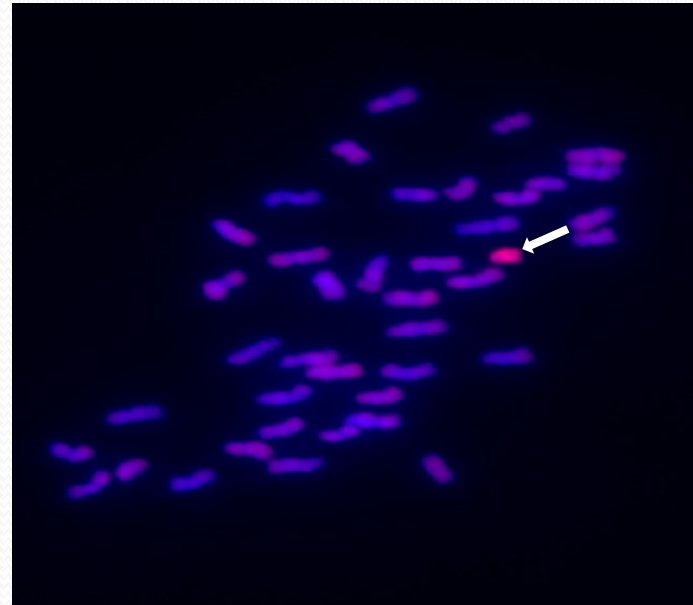
Screening of seeds of different crosses on the basis of *Th. elongatum* specific protein markers.



Genomic in situ Hybridization for the screening of *Thinopyrum elongatum* specific translocation in *Triticum aestivum*



Chromosomal translocation



Broken chromosome

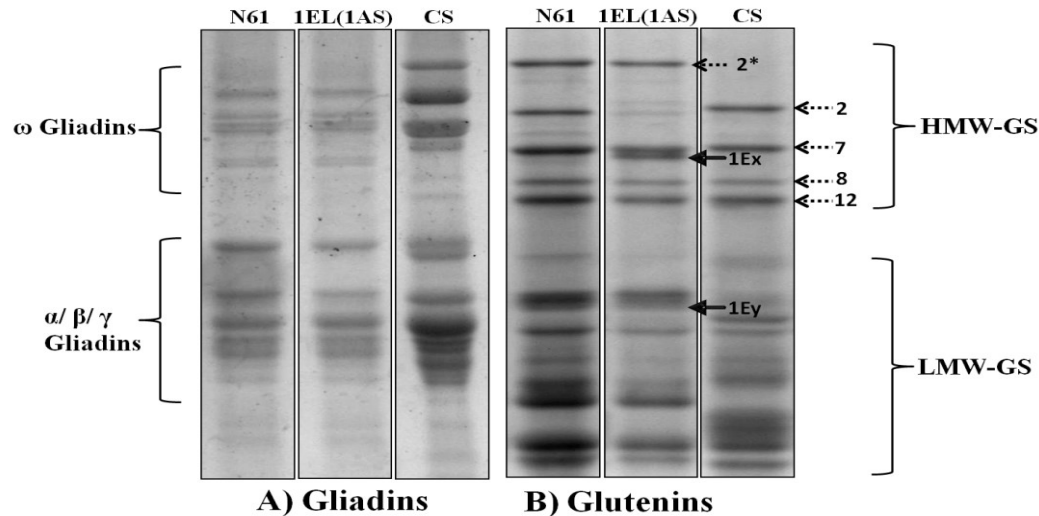
Frequency of generation of chromosome specific translocation [1EL(1AS)] in different crosses

Cross	No. of seeds analyzed	No. of seeds selected (SDS PAGE)	No. of seeds selected (GISH)	% age of translocated chromosome/screening efficiency
1E(1D) X N1AT1D X AcDomain X AcDomain	76	4	0	0
1E(1D) X N1AT1D X AcDomain X <i>Ph^I</i>	88	6	1	1.14/16.66
1E(1D) X N1AT1D X N61 X N61	20	3	1	5/33.3

Characterization of translocation line

Protein based

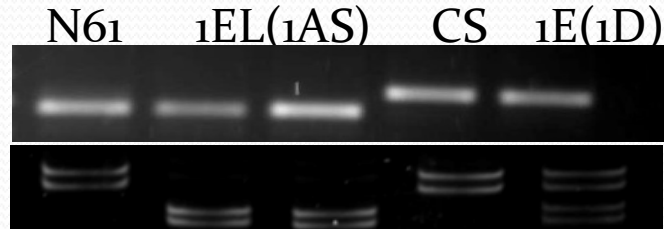
- *Th. elongatum* specific HMW-GS on SDS PAGE
- Gliadin profile to check for the presence of chromosome 1A coded gliadins and absence of *Th. elongatum* coded gliadins



Characterization of translocation line

SSR Markers based

- *Th. elongatum* specific bands identified using 1E(1D) substitution line
- BARC₁₇, WMC₄₆₉, WMC₃₁₂ and CFA₂₂₁₉
- WMC₄₆₉ near HMW-GS locus



N61 pattern-1AS marker

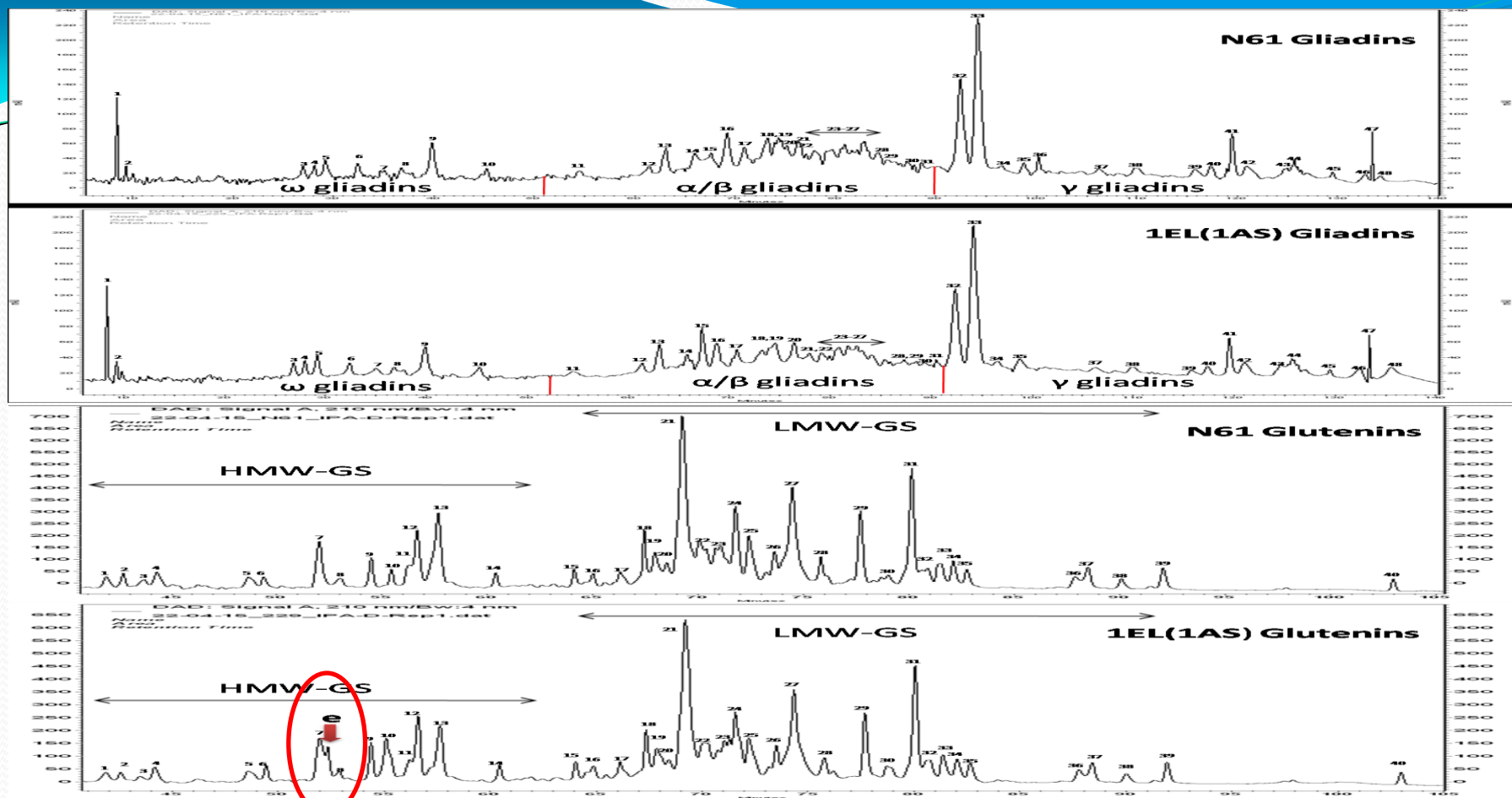
1E pattern- 1AL marker

C

1-0.17

0.47-0.61

0.61-1



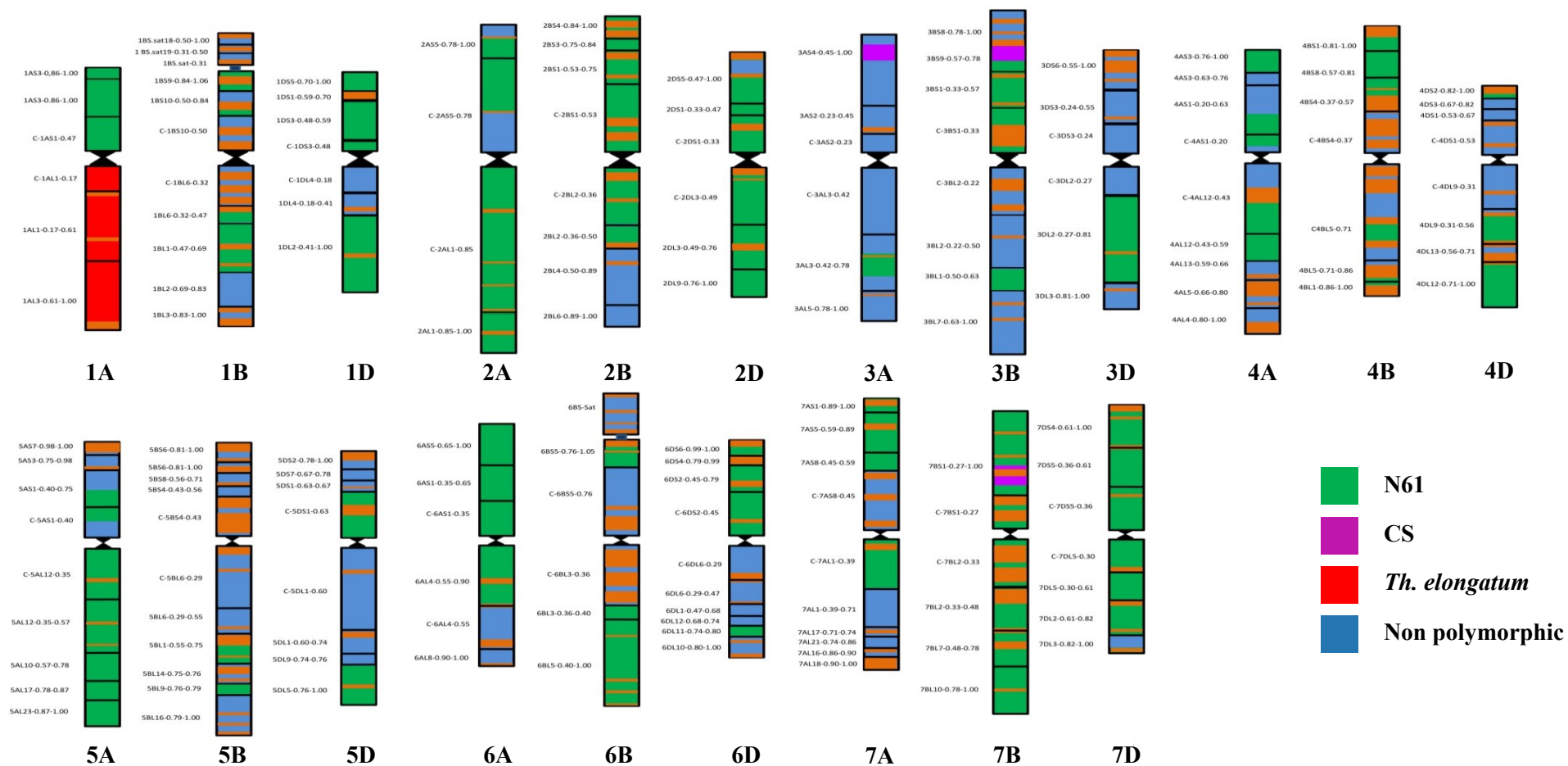
RP-HPLC profiles for gliadins and glutenins of DTL {1EL(1AS)} in comparison to N61

Background screening

- Total 536 deletion bin based primers specific to SSRs used
- BC_3F_4 (Line 1), BC_4F_3 (Line 2) were screened
- 119 polymorphic markers
- 85.7% showing pattern of background cultivar N61
- 2.5% CS pattern

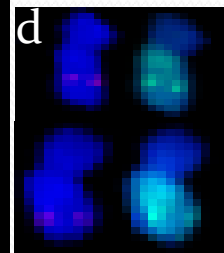
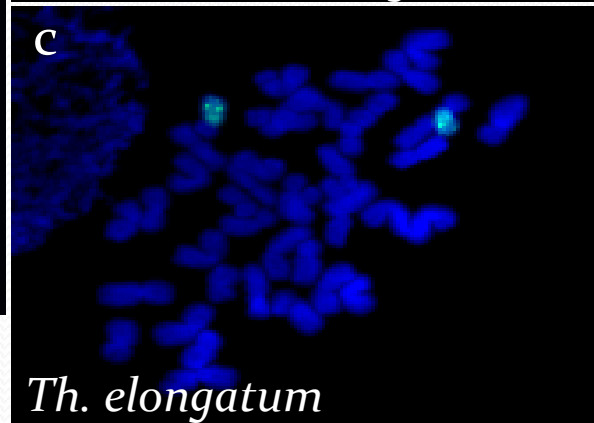
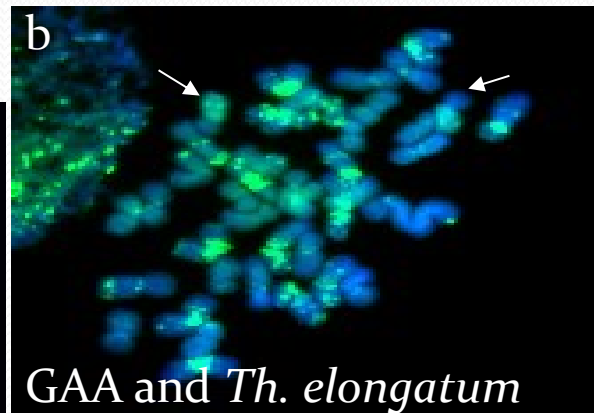
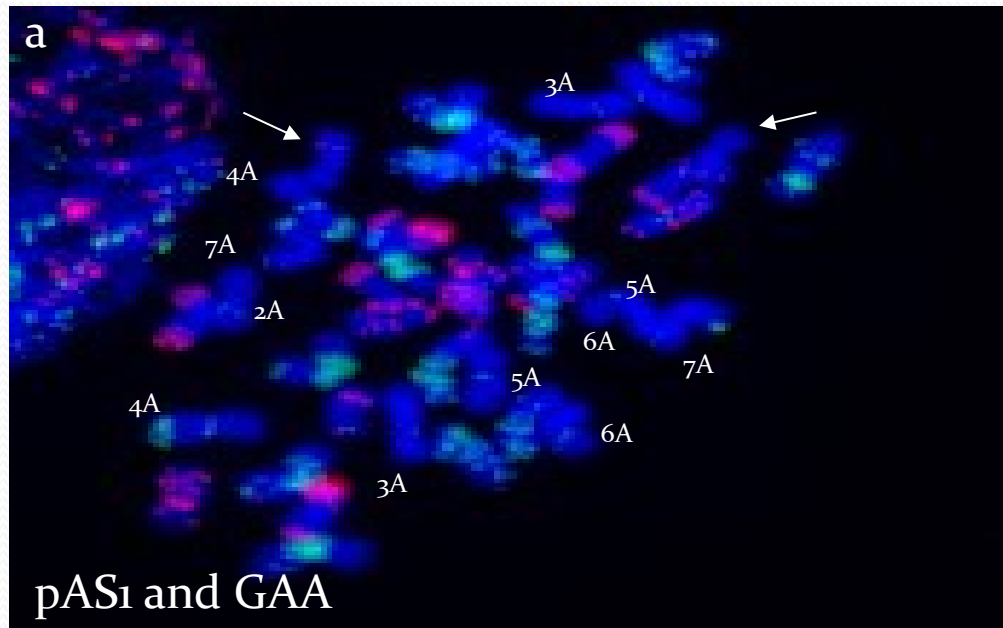
Table : SSR marker based screening of translocation line

Chromosome no.	SSR Primers	Polymorphic Markers	N61 Pattern	CS Pattern
1B	28	7	7	0
2A	27	6	6	0
2D	26	10	10	0
3B	24	6	5	1
4A	21	4	4	0
4D	20	2	2	0
5B	19	1	1	0
6A	30	6	6	0
6D	25	5	0	0
7B	26	9	9	1



Schematic microsatellite marker based map of 1EL(1AS) translocation lines

Cytological analysis

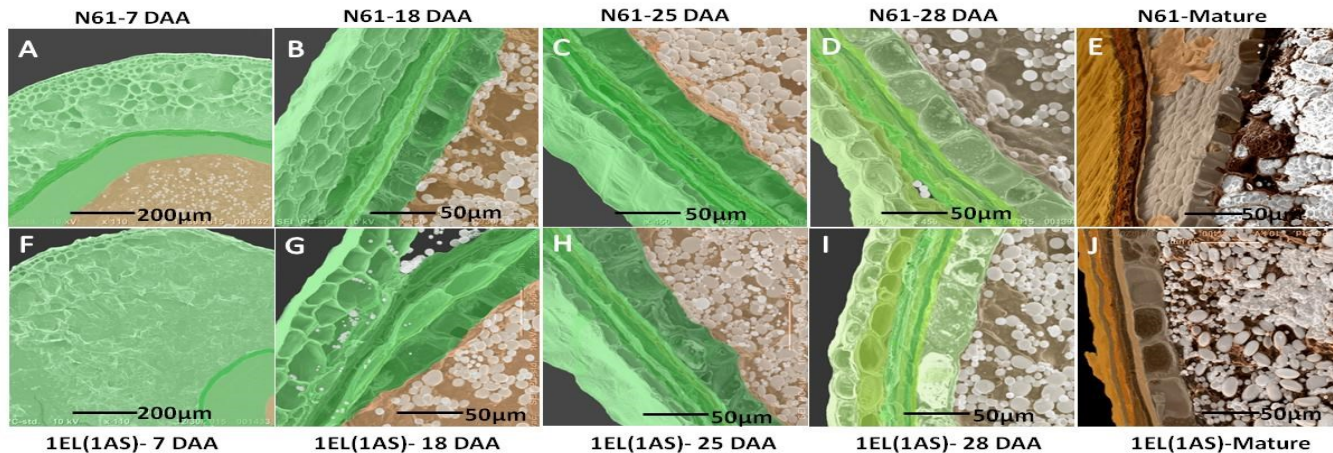


GAA and *Th. elongatum*
pAS1 and
GAA

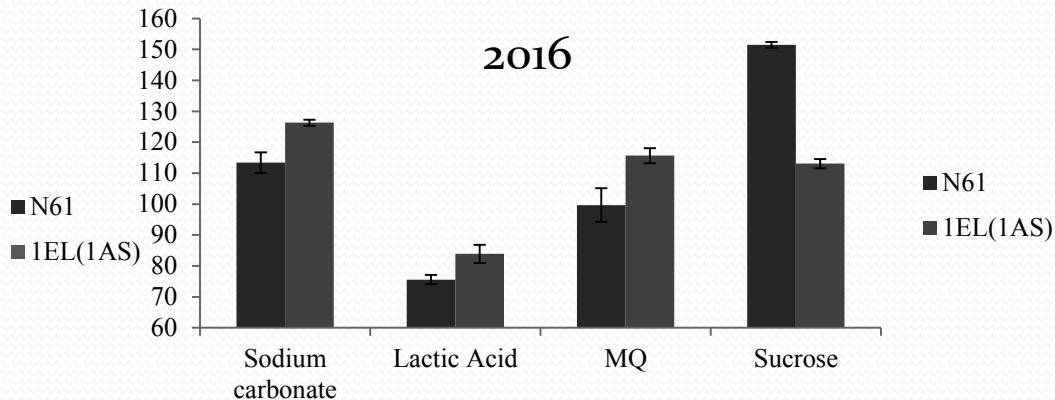
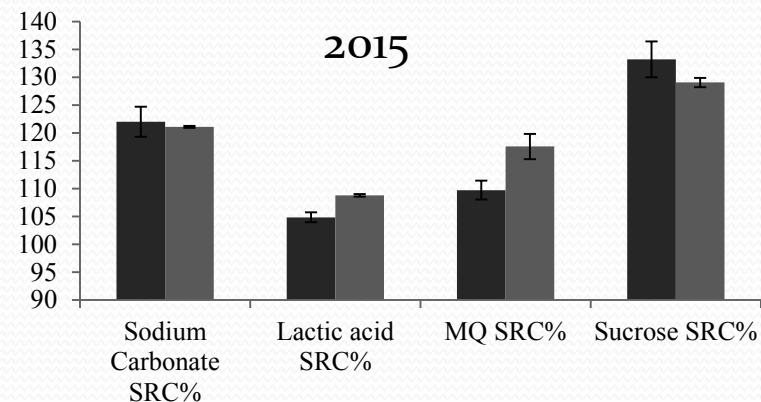
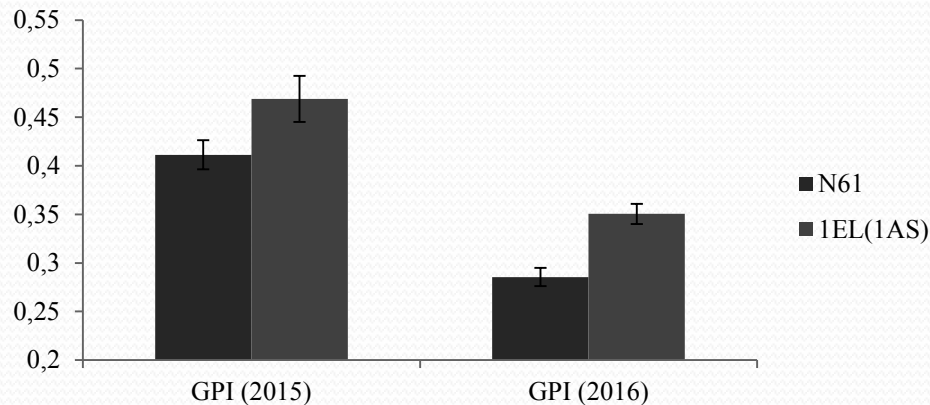
Morphological Similarities



Parameters	Norin61	1EL(1AS)
Plant height (cm)	102.6 \pm 5.42 ^a	104.67 \pm 4.58 ^a
Spike length (cm)	8.5 \pm 0.65 ^a	9.3 \pm 0.96 ^b
Awn length (cm)	4.11 \pm 0.78 ^a	3.89 \pm 0.89 ^a
Spike lets	18 \pm 2 ^a	18 \pm 2 ^a
Flag leaf length (cm)	17.79 \pm 3.73 ^a	18.92 \pm 2.23 ^a
Flag leaf width	1.49 \pm 0.28 ^a	1.51 \pm 0.18 ^a



Gluten performance index and Solvent retention capacity



a)

b)

Dough Extensibility and SDS Sedimentation Assay

- Increase in dough extensibility properties and SDS sedimentation values in 1EL(1As)

2015 Data

	Peak Positive Force (g)	Positive Area (g.sec)	Area To Positive Peak (g.sec)	Stretching Distance (mm)	SDS sedimentation test
1EL(1AS)	30.59±1.96 ^b	178.07±8.14 ^b	90.13±10.02 ^a	13.25±0.97 ^b	3±0.1 ^b

2016 Data

	Peak Positive Force (g)	Positive Area (g.sec)	Area To Positive Peak (g.sec)	Stretching Distance (mm)	SDS sedimentation test
1EL(1AS)	36.02±3.96 ^b	174.37±21.05 ^b	93.18±16.03 ^b	12.87±1.19 ^b	2.81±0.18 ^b

Quality Parameters

- No significant differences in quality parameters observed except for TKW values

2014 Data

	Protein content (%)	Dry Gluten (%)	Wet Gluten (%)	Thousand Kernel Weight (g)	Test Weight (g)	Grain Hardness
				35.38±0.63 ^a		
1EL(1AS)	10.65±0.44 ^a	8.4±0.72 ^a	22.67±2.54 ^a	32.15±0.49 ^b	74.6±0.71 ^a	30

2015 Data

	Protein content (%)	Dry Gluten (%)	Wet Gluten (%)	Thousand Kernel Weight (g)	Test Weight (g)	Grain Hardness
				33.63±0.76 ^a		
1EL(1AS)	8±0.3 ^a	5.9±0.3 ^a	17.2±0.6 ^a	30.47±2.33 ^b	74.9±0.5 ^a	35.15

2016 Data

	Protein content (%)	Dry Gluten (%)	Wet Gluten (%)	Thousand Kernel Weight (g)	Test Weight (g)	Grain Hardness
				34.67±0.51 ^a		
1EL(1AS)	8.87±0.61 ^a	5.03±0.67 ^a	17.63±1.40 ^a	28±3.28 ^b	77.077±0.68 ^a	33

Mixing properties

	Midline Peak Time (min)	Midline Peak value (%)	Midline peak width (%)	Midline peak integral (%tq*min)
1EL(1AS)	5.57±0.54 ^b	48.96±2.78 ^b	14.67±2.19 ^a	233.94±7.84 ^b

Conclusion...

- 1EL(1AS) translocation confirmed by GISH
- Morphological similarity to parent line
- Improved dough mixing properties
- No changes in Protein content, Grain hardness index, Dry gluten, Wet gluten and test weight
- Improved properties are due to chromosomal translocation

Publication:



Rapid Development and Characterization of Chromosome Specific Translocation Line of *Thinopyrum elongatum* with Improved Dough Strength

OPEN ACCESS

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