TISSUE CULTURE-INDUCED VARIABILITY

Critical issues that impact the evaluation and use of transgenic plants

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TISSUE CULTURE-INDUCED VARIABILITY

- aka somaclonal variation (SCV)
- common (universal?) in regenerated plants
- genetic and epigenetic in nature
- HERITABLE!!!
- Larkin and Scowcroft, TAG 60:197, 1981:

"…the failure to observe gross changes…does not negate the possibility of genetic variations which careful…analysis [would] reveal"

TISSUE CULTURE REDUCED BARLEY AGRONOMIC PERFORMANCE

Selected agronomic characteristics of lines derived from 10–12-wk-old callus, as measured in small-plot yield trials at three Idaho locations, 1992–1993.

Cultivar		Yield (# sig. dif. from control) (kg/ha)	Test weight ((kg/m^3)	Plump kernels (%)
Atlas 57	**Control**	**5859**	**595**	**88.9**
	6 R_2-derived families	4547–5128 (6)[a]	568–584 (6)	83.3–90.6 (3)
Golden Promise	**Control**	**6165**	**618**	**58.8**
	6 R_2-derived families	5612–6036 (2)	605–622 (2)	42.0–54.5 (3)
Klages	**Control**	**5859**	**640**	**62.6**
	4 R_2-derived families	4956–5379 (3)	609–649 (1)	45.4–61.2 (2)
Morex	**Control**	**5364**	**632**	**69.3**
	3 R_2-derived families	4929–5128 (0)	619–628 (2)	65.3–68.9 (1)
Piroline	**Control**	**6063**	**667**	**78.4**
	5 R_2-derived families	5208–5746 (2)	631–664 (3)	45.2–72.3 (3)
Steptoe	**Control**	**6923**	**597**	**82.5**
	6 R_2-derived families	6600–7036 (0)	588–601 (0)	80.4–83.5 (0)

[a]Number of families with means significantly different from the control for the specified trait.

TISSUE CULTURE REDUCED MALTING QUALITY OF BARLEY

Selected malting quality characteristics of lines derived from 10–12-wk-old callus, as measured in small-plot yield trials at two Idaho locations, 1992–1993.

Cultivar		Barley protein (%)	Malt extract (%)	Soluble/total protein (%)	Diastatic power (°ASBC)	α-amylase (DU)
Klages	**Control**	**12.7**	**77.0**	**36.2**	**109**	**39.5**
	4 R_2-derived families	13.2–14.0 (2)[a]	76.0–77.9 (1)	34.1–40.0 (1)	104–118 (0)	36.1–38.0 (1)
Morex	**Control**	**12.8**	**77.4**	**40.0**	**142**	**44.0**
	3 R_2-derived families	13.6–14.0 (2)	76.2–77.6 (1)	39.9–40.6 (0)	167–192 (3)	39.4–40.5 (2)
Piroline	**Control**	**12.2**	**76.7**	**34.6**	**115**	**34.4**
	5 R_2-derived families	13.2–13.7 (3)	74.6–76.7 (2)	28.5–35.0 (2)	107–139 (1)	28.7–35.8 (2)

[a]Number of families with means significantly different from the control for the specified trait.

TRANSFORMATION INDUCED ADDITIONAL VARIABILITY

Agronomic performance of Golden Promise T_2 transgenic-derived null-segregant barley (as a percentage of non-transgenic GP) grown at two locations in 194 in rows of spaced plants				
Family	# lines in family	Height	Yield	100-seed-weight
GP717B-2	1	88	56	74
GP717B-4	5	98	85	84
GP717B-11	2	86	54	70
GP717B-14	2	73	16	57
GP717B-31	1	79	47	77
GP717B-32	5	94	66	79
GP717B-33	4	90	64	74
GP717B-59	1	87	64	81
GP717B-189	4	77	27	66
GP717B-197	5	82	49	72
GP724B-1	1	87	45	74
GP724B-4	4	87	60	93
GP724B-47	1	92	79	88
GP724B-96	4	80	50	75

No recovery of performance with generation advance

IMPACT OF SCV ON GENETIC ENGINEERING

- commercially unacceptable performance
  - repair by breeding.....time!
  - one backcross took 69% of control yield to 94% in transgenic Conlon plants
- Effects of SCV and intended genetic alterations are confounded
  - qualitative traits: less of an issue
  - quantitative traits: big issue

SCV AFFECTS YOUR CHOICE OF CONTROL

- Popular choice: [illegible]tured parent

- Null-segregant(s) from same event

- Near-isogenic line

SCV IN TRANSGENIC WHEAT HAD LESS IMPACT

Agronomic performance of null-segregant transgenic wheat lines, as measured in small-plot yield trials in 2002 and 2003.

Location	Aberdeen, ID			Davis, CA		El Centro, CA	
Line	Yield (kg/ha)	Protein (%)	Test weight (kg/m^3)	Yield (kg/ha)	100-seed-weight (g)	Yield (kg/ha)	Test weight (kg/m^3)
Bobwhite	**6486**	**12.5**	**792**	**2481**	**3.34**	**9936**	**746**
Dx51D							
Hybrid-							
Hybrid-B null2	5974	13.1	802	2333	3.1	9323	766
LongDx5-B null	6476	12.9	796	1780	3.2	10126	766
LongDx5-F null	6226	13	795	1927	3.19	10095	775
LongDx5-H null	6217	13	793	2064	3.41	9983	759
LongDx5-I null	5982	12.9	792	1905	3.34	10735	775
ShortDx5-C null	6113	13.8	792	2599	3.31	9771	766
ShortDx5-D null	6597	13	787	2399	3.09	11078	756
ShortDx5-H null	6082	13.9	792	1928	3.21	10960	788*

*, **, ***: significant at P=0.05

20/30 yield < Bobwhite, mean = 6200 vs. 6301

but not NO impact

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