

Breeding Lettuce for Water and Nitrogen Use Efficiency

Next Generation
Breeding: Genes to
Growers

Specialty Crop
Research Initiative



Next Generation Breeding: genes to growers

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Why breed for water and nitrogen use efficiency?

- Increasing pressure to reduce water and nitrogen use
- *Goal:* Develop lettuce cultivars that produce economically sustainable yield and quality with less inputs of water and nitrogen

Outline

- Define the traits
 - Nitrogen Use Efficiency (NUE)
 - Water Use Efficiency (WUE)
- Review plant breeding methods for lettuce
- 2011 field experiment
- Future directions for research project
 - Genetic analysis
 - Cultivar development

Previous research: Abiotic stress tolerance.
Current research: Efficiency in irrigated
lettuce production



- GMO strategies for abiotic stress tolerance
 - Summarized by Reguera et al. 2012, *Biochimica et Biophysica Acta* 1819 :186–194
 - No examples with lettuce

Nitrogen Use Efficiency (NUE)

(Witecombe et al. 2008, Phil. Trans. R. Soc. B 363:703–716)

● Nitrogen use efficiency

● Two components

● Efficiency of N uptake from soil

= total plant N / soil N

● Efficiency of utilization

● Ability of plant to produce harvestable product

= harvestable biomass / total plant N

WUE: Water transpiration and carbon assimilation

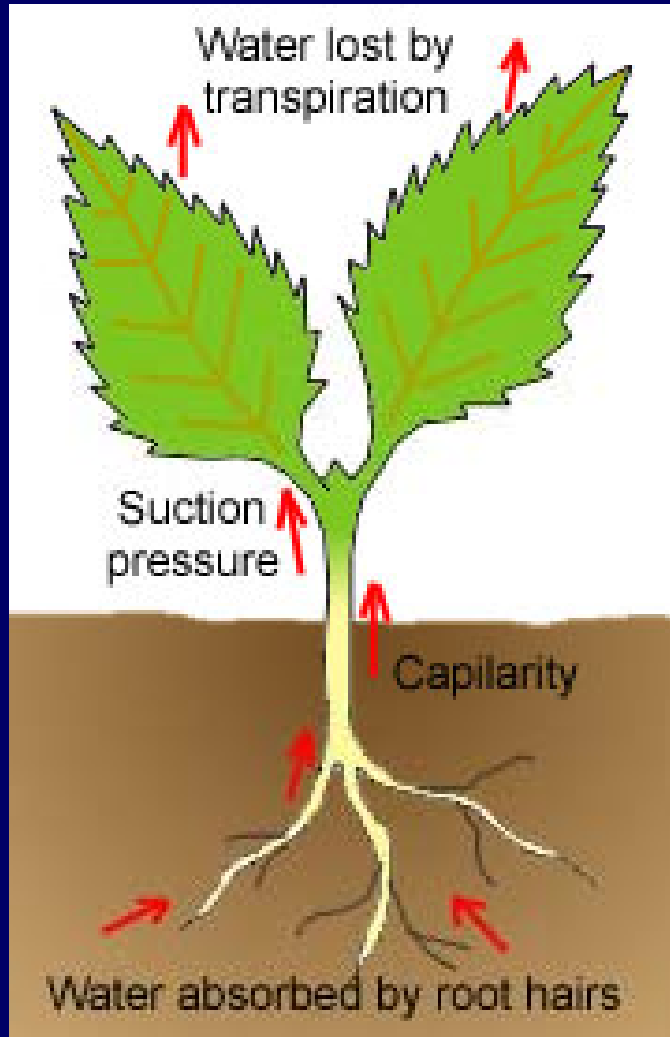
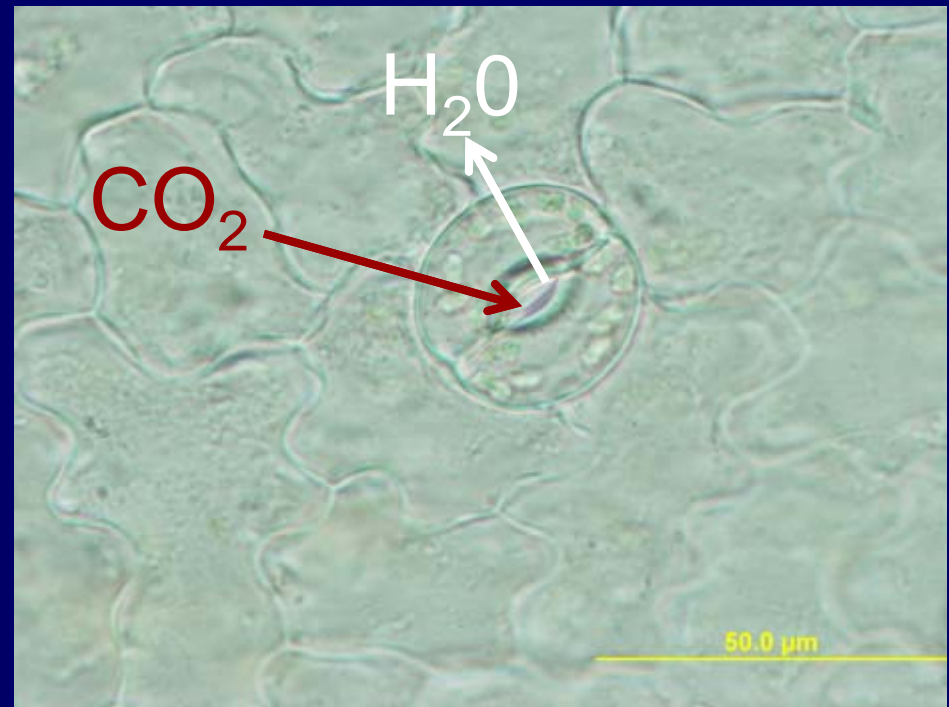


Image from www.scienceproject.com



Water Use Efficiency (WUE)

(Condon et al. 2004. *Journal of Experimental Botany*, 55:2247-2460

Morison et al., 2008. *Phil. Trans. Roy. Soc. B: Biol. Sci.* 363:639-658)

- Strategies for improving WUE in crops.
- Produce more biomass in exchange for water transpired by the crop (i.e., improving crop transpiration efficiency)
 - Examples of wheat cultivars in Australia
 - Generally coincides with a 'Conservative' growth strategy
- Uptake more of the available water
 - Reduce water wasted as evaporation from the soil surface, drainage beyond the root zone or being left behind in the root zone during harvest

roots



Image from:
<http://www.ars.usda.gov/is/pr/2007/070323.htm>



Image from:
<http://www.inra.fr/hyppz/IMAGES/7032601.jpg>

Components of plant breeding programs

Effective
selection
methods

Knowledge of crop
breeding systems

Genetic
variation

Lettuce, *Lactuca sativa* L.

- $2x=18$ (9 pairs of chromosomes)
- Compositae Family
- Self-pollinated, cultivars are inbred lines
- Genepool

L. sativa &

L. serriola (1°)

L. saligna (2°)

L. virosa (3°)



Lettuce: Breeding and Genetics Methods

- Breeding: pedigree method, backcrossing
- Genetics: F_2 , F_3 , recombinant inbred lines (RILs)



2011 field experiment



Objective: Determine the genetic variation for nitrogen use efficiency and water use efficiency

2011 field experiment

- Location: Spence Farm, Salinas, CA
 - Seeding Date: June 14
 - Followed cereal cover crop, very low residual N
- Crop Establishment
 - Overhead irrigation to germinate crop
 - 30 lbs N / acre pre-plant
- Treatments: Split-Split-Plot with four replications
 - Main plot: Drip irrigation (50% or 130% of crop ET)
 - Sub plot: Nitrogen post thinning (0 or 230 lb N/ acre)
 - Sub-sub plot: 50 lettuce accessions (all market types)

Field experiment layout

50% ETc	Low N	1	5	9	13	17	21	25	29	33	37	41	45	49	76	75
		2	6	10	14	18	22	26	30	34	38	42	46	50	78	55
		3	7	11	15	19	23	27	31	35	39	43	47	74	59	77
		4	8	12	16	20	24	28	32	36	40	44	48	56	73	79
	Std N	24	21	32	33	48	34	43	44	5	45	16	35	25	76	75
		29	42	38	12	19	9	47	17	8	1	23	50	40	78	55
		10	6	20	36	26	2	15	31	27	18	3	30	74	59	77
		39	7	4	13	49	22	41	11	37	14	28	46	56	73	79
130% ETc	Low N	2	43	13	21	22	8	42	14	25	50	23	10	38	76	75
		19	7	9	39	12	46	1	28	5	31	41	45	6	78	55
		40	36	47	16	3	11	33	48	15	27	34	17	74	59	77
		18	30	4	35	49	26	29	24	20	37	44	32	56	73	79
	Std N	13	42	11	38	30	46	10	31	34	41	27	23	14	76	75
		12	9	1	5	20	43	29	2	8	39	17	32	47	78	55
		24	19	33	26	3	36	48	22	16	28	45	25	74	59	77
		7	37	21	44	50	49	18	40	6	15	35	4	56	73	79

Lettuce accessions in 2011 NUE / WUE field experiment

Market type	Number of cultivar
iceberg	11
romaine	26
Batavia	2
butterhead	2
Latin	1
leaf	4
primitive / wild	4

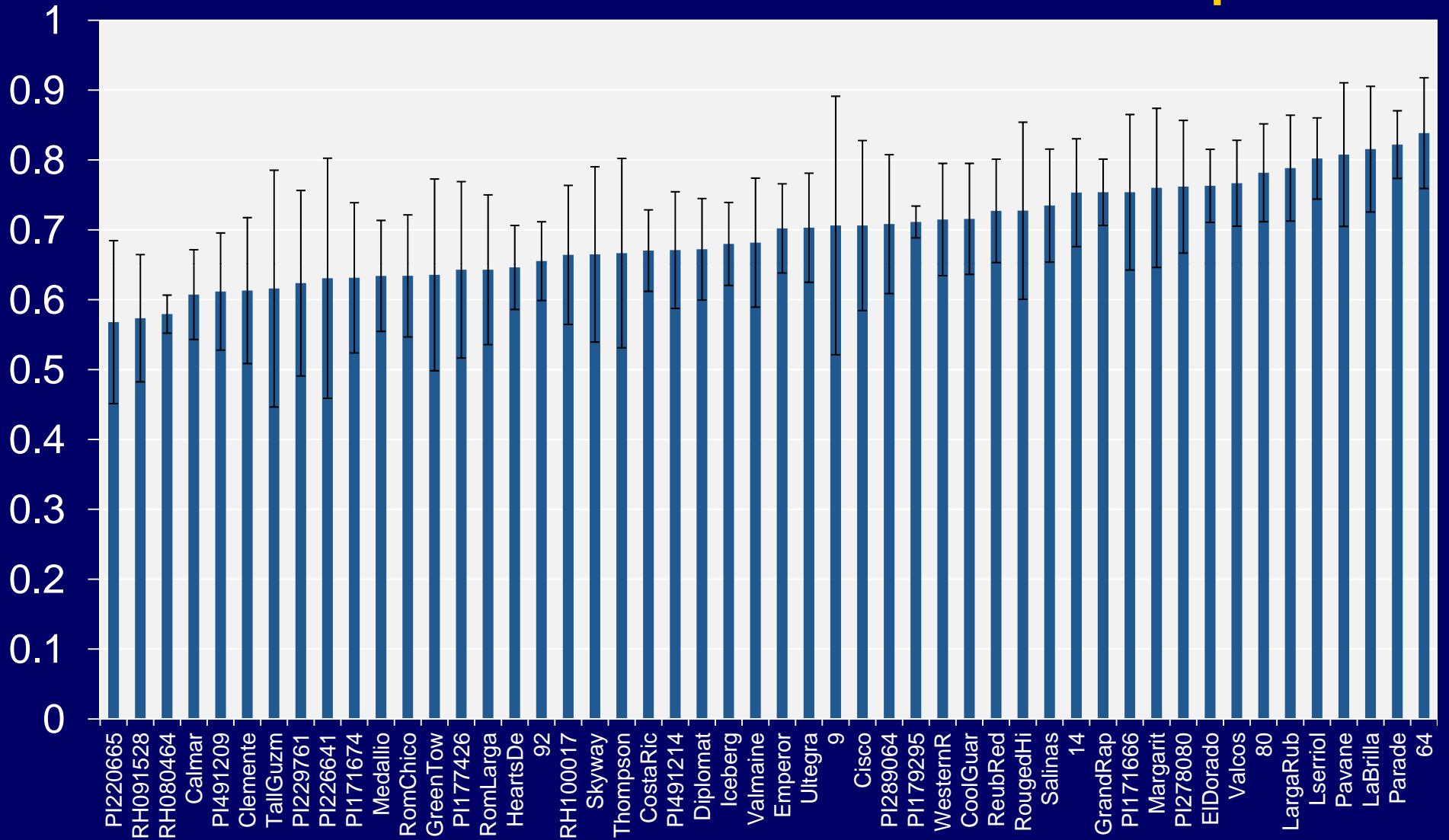
Variation in morphology: correlation with NUE and WUE



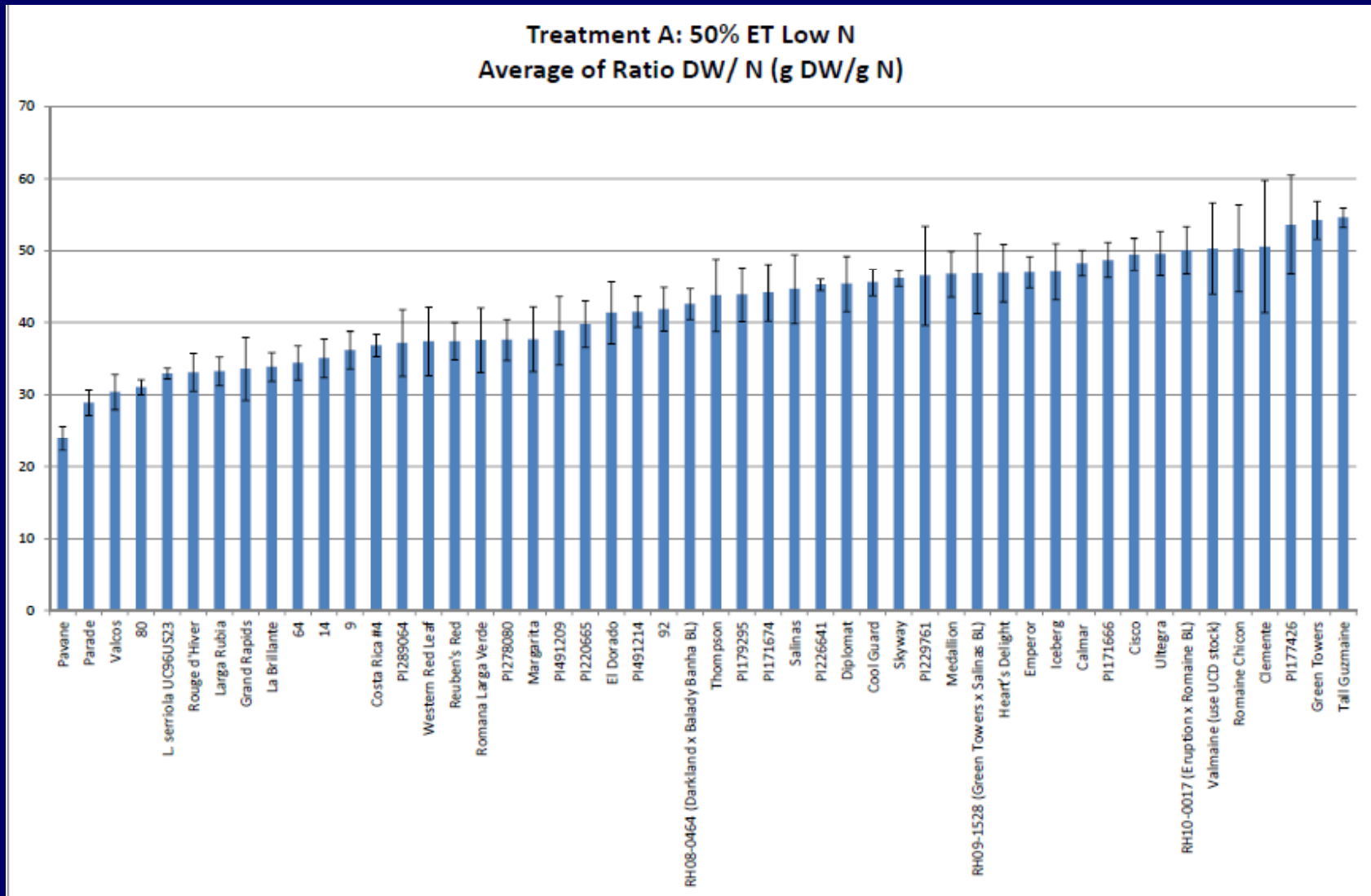
Variables measured

- Fresh Weight (g) and Dry Weight (g)
 - Calculate ratios with plant weight data
= Low input / standard input
- Plant total N, K, and P
 - Calculate N uptake and utilization
- Plant morphology: firmness, color, rate of development, leaf ruffle, leaf undulation, leaf savoy, head closure
- Data analyzed in SAS v 9.2
 - ANOVA
 - Calculated means and compared with LSD

Ratio of fresh wt: low water / standard input



Genetic variation for nitrogen utilization



What to do next

- Short-term (Miguel Gonzales)
 - Further data analysis of 2011 field experiment
 - Determine relationships between plant morphology and WUE / NUE
 - Determine if NUE / WUE phenotypes are repeatable over additional field experiments
 - Genetic analysis
 - Quantitative trait loci analysis with recombinant inbred line populations segregating for NUE or WUE
- Long term: breeding cultivars with increased NUE / WUE

Lettuce Genetic Resources for analyzing NUE and WUE

Population	1° Phenotypes	PD responsible	Status, Comments & Collaborators
Salinas x <i>L. serriola</i> UC96US23	Domestication traits, tipburn	RWM	Core inter-specific mapping population. 300 F ₈ RILs. Basis of ultra-high density transcript-based map. Gail Taylor, U. Southampton
	WUE & NUE	MC, RS, RWM	
Salinas x Valmaine	Tipburn	RWM, RJH, IS	Intra-specific, inter-type mapping population. 255 F ₇ RILs.
	Shelf-Life	RJH, YL	
Emperor x El Dorado	Tipburn	RWM	Collaboration with Sylvie Jenni, Ag Canada. 140 F ₇ RILs. Great Lakes & Vanguard iceberg types respectively. Emperor is tipburn-resistant.
Multiple <i>L. serriola</i> x <i>L. serriola</i> , <i>L. serriola</i> x <i>L. sativa</i> , & <i>L. sativa</i> x <i>L. sativa</i> crosses	Reduced symptoms to <i>Verticillium</i> race 2	RWM, RJH, IS, KS	
Diplomat x Margarita Green Towers x Western Red Leaf	Nutritional content: vitamins A, C & E, antioxidants, and folate	DWS	Diplomat (iceberg), Margarita (butterhead), 227 F ₈ RILs. Green Towers (romaine) x Western Red Leaf (red leaf), currently @ F ₄ .
Salinas 88 x La Brillante	Tipburn	RJH, RWM, IS	Intra-specific, intra-type mapping population. 95 F ₆ RILs. Segregates for race 1 resistance to <i>V. dahliae</i> , tipburn and shelf-life.
	Shelf-life	RJH, IS, YL	

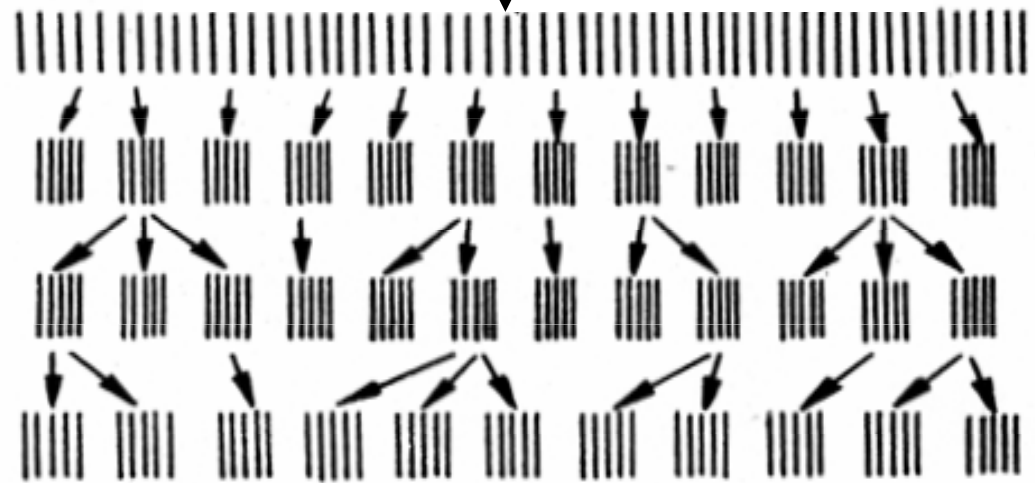
Cultivar Development

Genetic Diversity



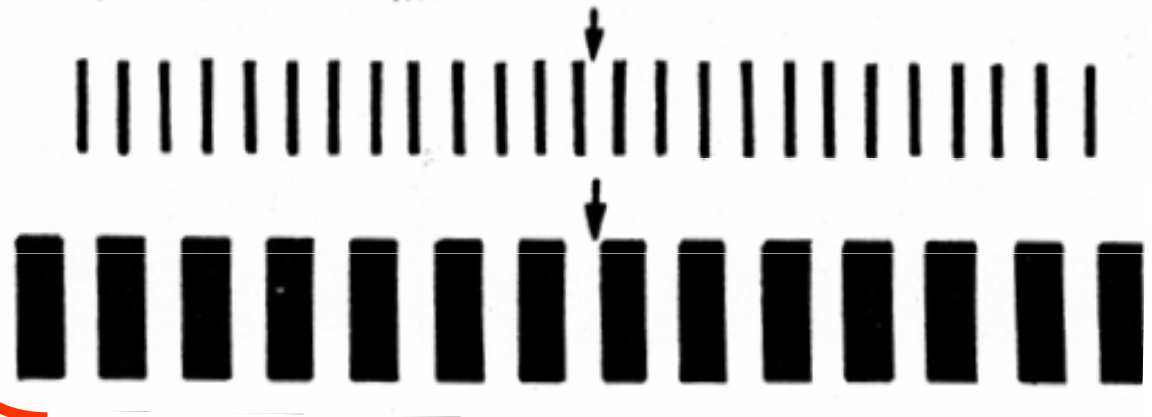
Early Generations

Selection a few
simply inherited
traits



Late Generations

Selection many
complex traits



Characterization of advanced breeding lines

(I. Simko, K. Subbarao, C. Bull, A. Fernandez, Y. Luo)

Resistance: lettuce drop, verticillium wilt, big vein, BLS, downy mildew, dieback, *LMV*

Yield & Quality: tipburn resistance, salad shelf-life, arrival quality



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