



# Enhancing Gains in Forage Breeding Using Clonal Replicates And Molecular Markers: A simulation study

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- Introduction
- Clonal selection
- Marker-based selection
- Conclusion



- **Forage breeding**
  - Many forage crops are obligate out-breeders, can be propagated vegetatively and show significant inbreeding depression.
  - Recurrent selection for general combining ability is the major method used to increase the mean performance of the improved population.
  - Synthetic cultivars developed by random mating between elite clones are the delivery system to transfer genetic gain to pastures.
  - Half-sib or full-sib progeny tests
  - Synthetic cultivars with 5-20 parents (clones)



- **Simulation**
  - More realistic genetic models can be used
  - Many testing and selection schemes can be investigated
  - The same model and selection scheme can be run many times
  - Better breeding strategy can be identified by investigating many combinations of the efficiency-affecting factors



# Clonal selection: Theory

Heritability of trait 2 (spaced plant)

$$h_2 = \frac{\sigma_{g2}^2}{\sigma_{g2}^2 + \sigma_w^2}$$

Heritability of trait 1 (clonal mean)

$$h_1 = \frac{\sigma_{g1}^2}{\sigma_{g1}^2 + \sigma_w^2 / r}$$

Heritability of trait 3 (target trait, sward performance)

$$h_3 = \frac{\sigma_{g3}^2}{\sigma_{g3}^2 + \sigma_{w3}^2}$$



# Clonal selection: Theory

Selection response of trait 3 (target trait) by selecting for trait 3 based on individual performance is

$$\Delta G_3 = i_3 h_3^2 \sigma_{p3}$$

Indirect selection response of trait 3 (target trait) by selecting for trait 2 is

$$\Delta G_{3,2} = i_2 r_{g23} h_2 h_3 \sigma_{p3}$$

Indirect selection response of trait 3 (target trait) by selecting for trait 1 (clonal mean) is

$$\Delta G_{3,1} = i_1 r_{g13} h_1 h_3 \sigma_{p3}$$



# Clonal selection: Theory

- Using clonal replicates can increase heritability ( $h_1$ )

$$h_1 > h_2$$

- Under fixed resource, using clonal replicates means that the number of clones (individuals) tested has to be reduced. Thus,

$$i_1 < i_2$$

- Can clonal replicates increase genetic correlations between selection criterion and objective?

$$r_{g23} > r_{g13} \quad ? \text{ (very likely)}$$



# Clonal selection: Theory

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- Clonal selection will be more efficient than spaced plant selection for forages
  - Heritability of most traits are low
  - Testing capacity in terms of number of plants is high
  - Clonal mean might be more closely correlated with the performance under sward condition.

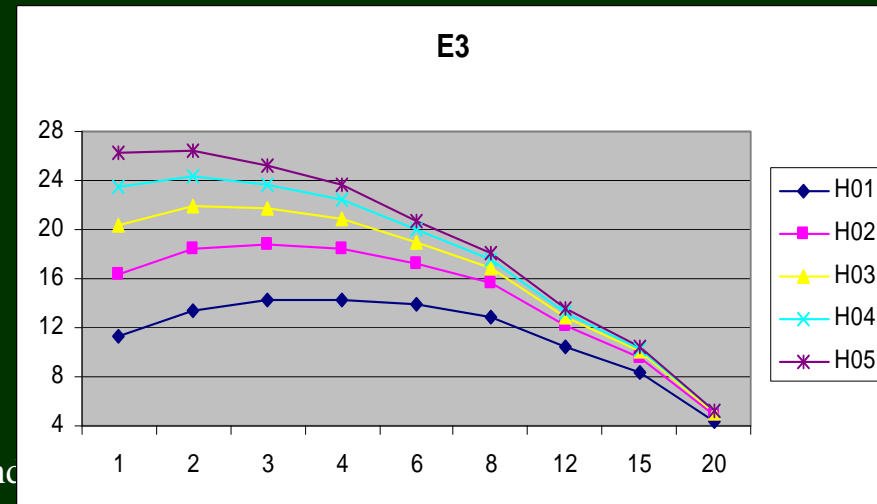
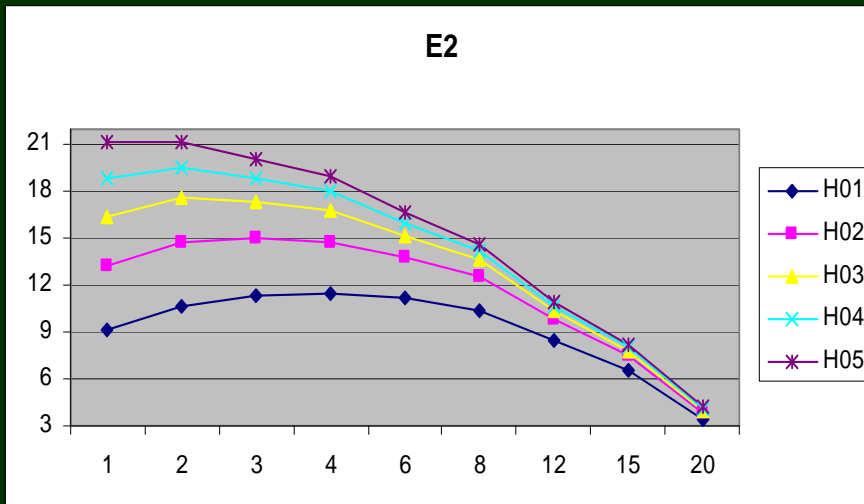
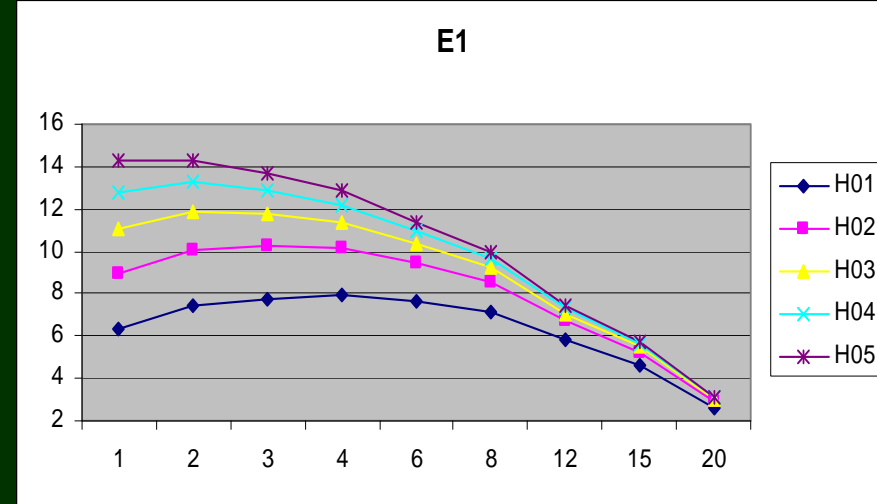
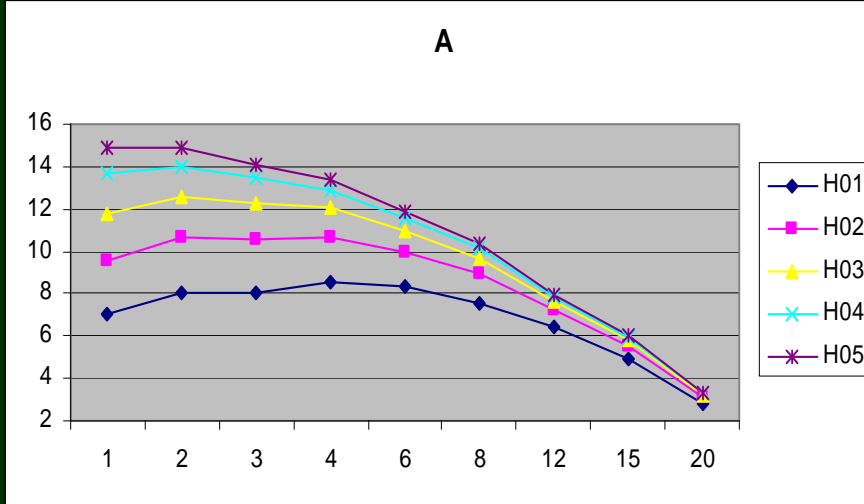


# Simulation

- The trait is controlled by 60 independent biallelic loci with gene frequency of 0.5.
  - Four gene action modes 1) additive model (A), all genes are purely additive; 2) two-gene epistasis (E1), the 60 genes are in 30 two-gene epistasis networks; 3) three-gene epistasis (E2), the 60 genes are in 20 three-gene epistasis networks and 4) four-gene epistasis (E3), the 60 genes were are in 15 four-gene epistasis networks.
  - The individual-based broad-sense heritability is another simulation factor with five (0.1, 0.2, 0.3, 0.4 and 0.5)
  - The effects of the genes for the A model were randomly sampled from a uniform [0, 1] distribution. The genotypic values of all possible genotypes for each gene network were randomly sampled from an uniform [0, 2], [0, 3] and [0,4] distribution for the E1, E2 and E3 models respectively.
- Assuming that there are 600 (2400) genotypes available and 24 genotypes are to be selected to be used in breeding

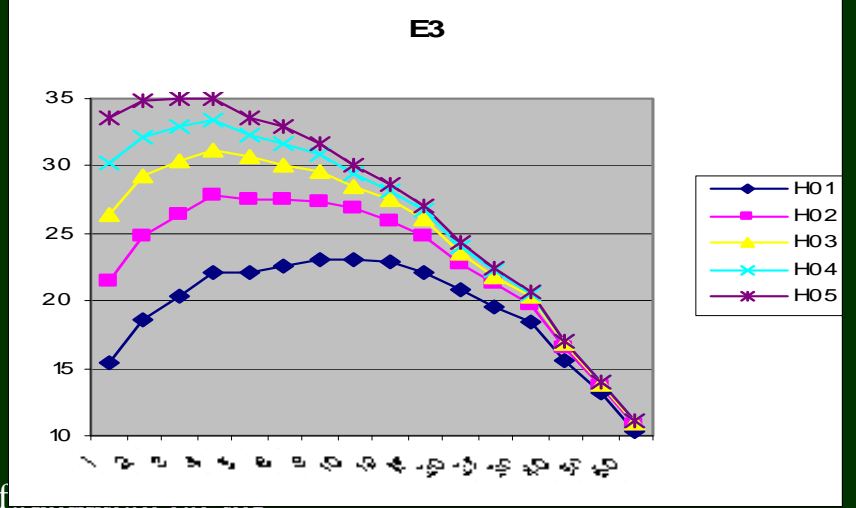
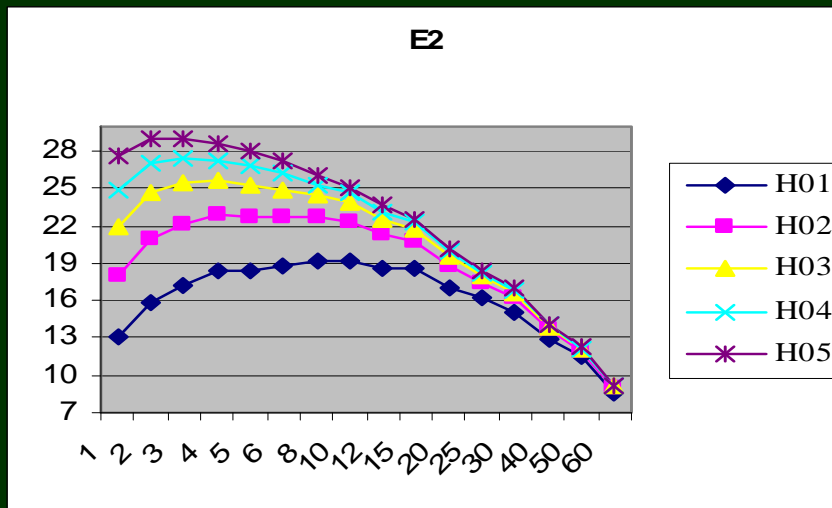
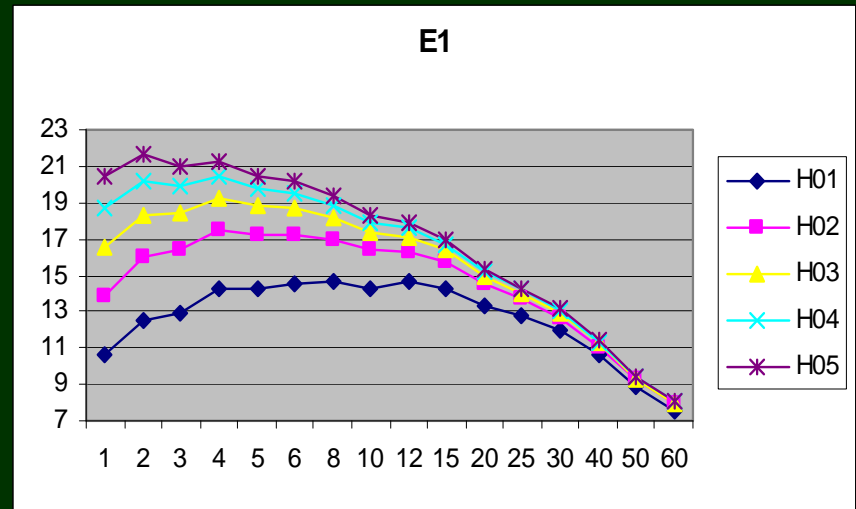
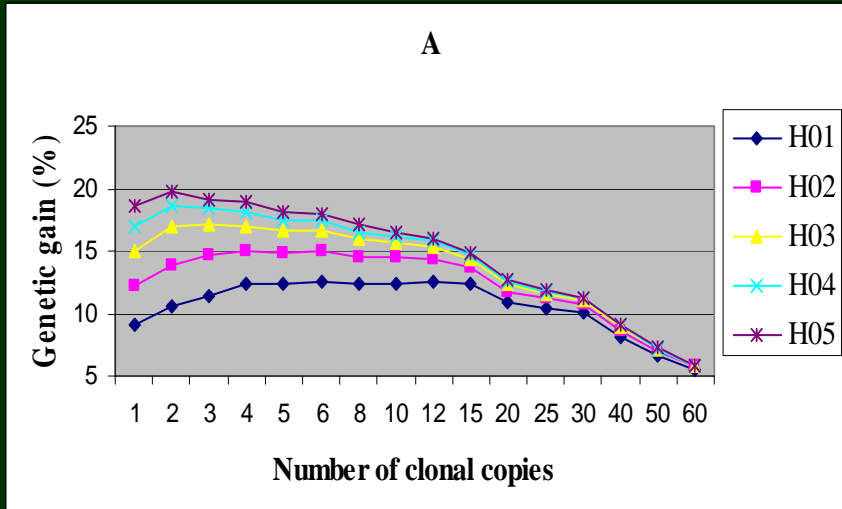


# Clonal Selection (600)





# Clonal Selection (2400)





- CS is very effective under all genetic models
- More clonal copies are needed for traits with low heritability.
  - A few copies per clone (5-6) are usually enough
  - The optimal number of copies/clone increases with testing capacity
- CS is more effective when testing capacity is high
  - The effect of reduced selection intensity caused by the use of clonal replication is small.



# Marker-based selection: Theory

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Selection response of the target trait by selecting for known genes (through linked markers)

$$\Delta G = i r_g h \sigma_p = i P E G h \sigma_p$$

$r_g$  = genetic correlation between the trait and a pseudo-trait controlled by the known-gene

PGE: Percentage of genetic variance accounted for by the known-gene



# Two-stage selection

- Using markers to pre-screen the population to reduce the number of genotypes to be field tested (MBS + PS): genotyping is less expensive

$$\Delta G = i_1 PEGh\sigma_p + i_2 h\sigma_p$$

- Selection based on individual performance is conducted first to reduce the number of genotypes to be genotyped (PS+MBS): phenotyping is less expensive

$$\Delta G = i_1 h\sigma_p + i_2 PEGh\sigma_p$$



# Two-stage selection

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- Pre-screen the population using markers and then clonal replicates are used to increase genetic gain from the second stage

$$\Delta G = i_1 PEGh\sigma_p + i_2 h_c\sigma_p$$



# Simulation

- Additive model is assumed.
- The number of genes affecting the trait is set at 42 (two QTL + 40 genes), 45 (5 QTL + 40 genes), 50 (10 QTL + 40 genes) and 35 (15 QTL + 20 genes) to generate four levels of genotypic variance accounted for by the QTL (PGV: 1/6, 1/3, 1/2, 3/4).
  - All the QTL have equal effect and all the other genes also have equal effect. The effect of QTL is twice of the other genes.
- Other simulation factors of the GE systems were
  - 3 levels of recombination rates between the QTL and its closest marker (0.00, 0.01, 0.05 or 0.100).
  - 3 levels of heritability (0.1, 0.3, or 0.5).
- MBS is conducted by using the marker score defined as the number of desirable marker alleles.



# MBS + PS

R15													
STR	MBS%	H01L00	H01L01	H01L05	H01L1	H03L00	H03L01	H03L05	H03L1	H05L00	H05L01	H05L05	H05L1
PS	100	7.13	6.75	6.67	6.39	12.58	12.78	12.77	11.99	16.90	16.63	16.78	16.33
MBSPS1	80	8.32	8.17	8.01	8.04	12.94	13.30	13.11	12.65	16.76	17.03	16.89	16.58
MBSPS2	60	9.85	9.84	9.53	9.68	13.90	14.14	13.80	13.44	17.34	17.45	17.03	16.82
MBSPS3	40	10.35	10.04	9.98	9.13	14.18	14.35	14.20	13.19	16.91	16.67	17.04	15.88
MBSPS4	20	11.27	11.38	10.97	10.60	14.03	14.47	13.80	13.09	16.73	16.76	16.00	15.43
MBS	4.17	10.63	10.35	8.86	8.67	10.63	10.35	8.86	8.67	10.63	10.35	8.86	8.67
R30													
PS	100	7.088	7.396	7.528	8.212	12.816	13.072	13.176	13.684	16.3	16.668	16.42	17.284
MBSPS1	80	8.348	8.14	8.304	8.576	12.844	13.1	12.816	12.764	16.86	16.88	16.672	16.732
MBSPS2	60	9.456	9.412	8.996	8.532	13.744	13.756	13.328	12.984	16.764	16.552	16.62	16.816
MBSPS3	40	10.624	10.424	10.124	9.94	14.072	14.368	13.984	13.708	16.52	17.076	16.384	16.6
MBSPS4	20	11.624	11.668	11.172	10.936	14.384	14.94	14.116	13.248	16.752	17.256	15.9	15.284
MBS	4.17	12.46	12.208	10.712	10.348	12.46	12.208	10.712	10.348	12.46	12.208	10.712	10.348
R50													
PS	100	7.22	7.17	7.00	7.42	12.51	12.60	12.79	12.97	15.26	16.18	16.68	16.34
MBSPS1	80	8.43	8.26	8.37	8.11	12.99	13.17	12.86	12.73	15.96	16.37	16.94	16.23
MBSPS2	60	9.34	9.40	10.14	9.19	12.74	12.94	13.36	13.36	15.73	16.33	16.87	16.66
MBSPS3	40	11.19	10.53	10.76	10.35	14.25	14.20	14.08	13.63	16.40	16.86	16.96	16.32
MBSPS4	20	12.80	12.78	12.52	11.54	15.26	15.42	14.91	13.98	16.96	17.14	16.70	15.92
MBS	4.17	15.32	15.22	13.55	12.19	15.32	15.22	13.55	12.19	15.32	15.22	13.55	12.19
R75													
PS	100	7.82	8.09	8.25	8.71	14.02	14.02	14.97	14.98	18.7	18.69	18.69	19.73
MBSPS1	80	10.55	10.82	10.23	10.02	15.36	15.37	15.16	15.77	19.37	19.30	18.21	19.20
MBSPS2	60	11.94	11.74	11.85	11.03	15.59	15.54	16.17	16.50	19.11	19.20	18.97	20.40
MBSPS3	40	13.6	13.51	13.51	14.11	16.74	16.93	17.14	17.64	19.64	19.49	19.41	21.00
MBSPS4	20	17.54	17.70	16.36	15.15	19.42	19.58	19.12	17.92	21.16	21.29	21.08	20.68
MBS	4.17	22.76	22.19	21.34	19.40	22.76	22.19	21.34	19.40	22.76	22.19	21.34	19.40

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# PS +MBS

R15													
STR	PSSP%	H01L00	H01L01	H01L05	H01L10	H03L00	H03L01	H03L05	H03L10	H05L00	H05L01	H05L05	H05L10
MBS	100	10.19	10.23	9.33	8.95	11.62	10.23	9.33	8.95	11.18	10.23	9.33	8.95
PSMBS1	80	11.06	10.53	10.18	8.33	11.68	11.15	10.80	9.08	11.76	11.25	10.56	9.04
PSMBS2	60	12.49	10.30	9.70	9.27	13.94	11.27	10.73	10.16	14.50	11.80	11.62	10.60
PSMBS3	40	11.17	12.10	11.67	10.55	14.07	13.90	13.23	12.07	15.81	13.94	13.61	13.28
PSMBS4	20	10.46	10.62	10.42	9.46	10.46	13.88	13.48	12.57	10.46	15.53	14.95	14.28
PS	4.17	7.60	7.45	7.84	7.13	13.06	13.03	13.29	12.80	16.15	15.88	16.56	16.20
R30													
MBS	100	13.09	12.76	11.852	9.976	13.03	12.76	11.852	9.976	13.10	12.76	11.852	9.976
PSMBS1	80	13.06	12.8	11.8	10.948	13.72	13.06	12.368	11.64	13.37	13.032	12.336	11.128
PSMBS2	60	13.54	13.448	12.58	11.352	14.90	13.696	13.256	12.112	15.33	13.532	13.164	12.484
PSMBS3	40	12.78	13.18	12.564	11.188	15.43	14.772	14.08	12.66	16.92	15.388	14.724	13.8
PSMBS4	20	12.76	12.552	11.808	12.252	12.76	15.14	13.904	14.12	12.76	16.432	15.868	15.54
PS	4.17	7.20	7.152	8.04	7.848	12.24	11.908	12.556	12.596	16.02	16.08	16.328	16.528
R50													
MBS	100	15.68	15.71	14.19	12.41	15.87	15.71	14.19	12.41	15.76	15.71	14.19	12.41
PSMBS1	80	15.72	15.82	14.22	12.65	16.11	15.87	14.33	12.89	16.27	15.74	14.25	12.92
PSMBS2	60	15.50	15.28	14.26	12.54	16.89	16.23	14.93	13.19	16.96	15.97	14.92	13.56
PSMBS3	40	14.61	15.47	13.89	12.78	16.78	16.76	15.17	13.95	17.35	17.43	15.53	14.45
PSMBS4	20	15.94	13.94	13.09	12.10	15.94	16.45	15.42	14.36	15.94	17.51	16.23	15.61
PS	4.17	6.62	6.72	6.49	5.77	11.91	11.88	11.73	11.13	14.99	15.33	15.30	14.74
R75													
MBS	100	23.01	22.77	20.66	18.38	23.54	22.77	20.66	18.38	23.51	22.77	20.66	18.38
PSMBS1	80	22.42	22.88	20.72	19.15	22.72	22.88	21.07	19.33	22.93	23.00	21.02	19.31
PSMBS2	60	22.05	21.98	20.61	19.04	23.40	22.29	21.21	19.19	23.40	22.59	21.00	19.19
PSMBS3	40	18.83	21.78	19.69	18.37	21.88	22.89	20.98	19.36	22.88	23.59	21.28	20.27
PSMBS4	20	23.04	18.81	17.86	15.12	23.04	21.47	20.98	18.81	23.04	22.67	22.03	20.13
PS	4.17	7.81	8.83	8.22	8.29	14.26	14.43	14.61	14.47	18.52	18.90	18.11	19.26

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## R15

STR	MBSSP(%)	No. Copies	H01L00	H01L01	H01L05	H01L10	HO3L00	H03L01	H03L05	H03L10	H05L00	H05L01	H05L05	H05L10
PS	100	1	7.36	7.33	7.75	7.91	12.27	12.70	12.60	12.63	16.41	16.39	16.33	16.00
MBSCS1	50	2	11.59	11.61	10.96	11.48	16.30	16.72	15.99	16.15	19.13	19.15	18.69	18.83
MBSCS2	33.33	3	13.43	12.67	13.15	12.86	17.40	17.47	17.47	16.81	19.48	19.37	19.53	18.91
MBSCS3	25	4	13.88	13.85	14.35	13.51	17.99	18.04	18.04	17.07	19.73	19.59	19.65	18.97
MBSCS4	20	5	14.80	14.26	13.48	13.42	17.70	18.40	17.10	16.64	19.28	19.30	18.63	18.10
MBSCS5	16.67	6	16.03	15.19	14.51	14.51	18.60	18.37	18.29	17.33	19.50	19.23	19.19	18.53
MBSCS6	12.5	8	15.89	15.54	15.47	14.77	18.14	18.25	17.38	16.82	18.98	18.83	18.30	17.66
MBSCS7	10	10	16.06	16.37	15.42	13.90	17.97	18.20	17.29	15.77	18.55	18.67	17.81	16.35
MBSCS8	8.33	12	14.97	15.28	13.73	13.77	16.43	16.69	15.28	14.88	16.87	17.07	15.73	15.49
MBSCS9	6.67	15	14.20	14.04	13.57	12.31	15.20	15.19	14.59	13.13	15.52	15.44	14.96	13.48
MBSCS10	5	20	12.48	12.31	12.10	11.37	12.90	12.76	12.38	11.65	12.78	12.85	12.48	11.76

## R30

PS	100	1	7.07	7.01	7.36	7.13	12.58	12.82	12.01	12.10	16.48	16.81	16.28	16.29
MBSCS1	50	2	12.33	11.86	11.52	11.48	16.43	16.68	16.15	16.02	19.18	19.86	19.30	19.41
MBSCS2	33.33	3	14.12	14.18	14.11	13.56	17.77	18.08	17.79	17.56	19.97	20.22	19.90	19.82
MBSCS3	25	4	15.08	14.58	14.66	14.15	19.02	18.78	18.45	17.74	20.70	20.36	20.15	19.79
MBSCS4	20	5	15.33	15.57	15.28	14.58	18.30	18.66	18.22	17.36	19.51	19.84	19.72	18.82
MBSCS5	16.67	6	17.07	16.72	16.48	15.23	19.90	19.80	19.02	17.58	21.05	20.87	20.15	18.94
MBSCS6	12.5	8	16.92	16.46	16.04	15.41	19.38	19.17	18.69	17.36	20.15	20.04	19.43	18.36
MBSCS7	10	10	16.64	16.27	15.13	14.93	18.24	18.05	16.95	16.44	18.82	18.61	17.55	17.02
MBSCS8	8.33	12	16.78	16.24	15.49	15.10	18.27	17.72	17.00	16.24	18.59	18.00	17.37	16.78
MBSCS9	6.67	15	15.69	15.78	15.18	14.22	16.68	16.58	15.84	15.04	16.91	16.84	16.08	15.32
MBSCS10	5	20	15.17	15.14	14.00	12.62	15.50	15.59	14.37	12.99	15.63	15.67	14.50	13.12

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							R50							
STR	MBSSP(%)	No. Copies	H01L00	H01L01	H01L05	H01L10	H03L00	H03L01	H03L05	H03L10	H05L00	H05L01	H05L05	H05L10
PS	100	1	7.06	6.94	6.00	7.02	12.16	12.12	11.49	11.66	15.39	15.88	15.41	15.47
MBSCS1	50	2	11.16	11.35	11.46	11.47	15.45	15.45	15.79	16.02	17.62	17.95	18.40	18.73
MBSCS2	33.33	3	13.72	13.58	13.53	13.42	17.58	17.48	17.23	17.64	19.17	19.33	19.49	19.24
MBSCS3	25	4	16.02	15.73	14.92	14.27	19.16	18.97	18.76	17.51	20.33	20.47	20.11	19.07
MBSCS4	20	5	15.61	15.77	15.76	14.99	18.63	18.74	18.72	18.48	19.85	19.81	19.82	19.63
MBSCS5	16.67	6	16.51	16.48	15.66	15.41	19.14	18.97	18.34	18.01	20.05	20.09	19.42	19.16
MBSCS6	12.5	8	17.25	17.01	16.61	16.25	19.57	19.24	19.00	18.50	20.11	19.97	19.73	19.10
MBSCS7	10	10	17.50	17.45	16.68	15.91	19.26	19.16	18.50	17.78	19.88	19.79	18.96	18.23
MBSCS8	8.33	12	17.71	18.02	16.24	16.01	18.96	19.23	17.84	17.36	19.40	19.66	18.26	17.93
MBSCS9	6.67	15	17.71	17.45	16.22	15.44	18.74	18.40	17.27	16.65	19.02	18.73	17.52	16.90
MBSCS10	5	20	16.97	16.58	15.20	14.22	17.35	16.90	15.59	14.71	17.45	17.00	15.71	14.79
							R75							
PS	100	1	9.10	8.96	8.23	8.48	14.14	13.78	13.99	14.76	18.52	18.53	18.15	19.48
MBSCS1	50	2	14.88	14.40	14.08	13.93	19.92	19.04	19.31	18.79	22.99	22.37	22.00	22.42
MBSCS2	33.33	3	17.45	17.09	17.04	17.09	21.32	20.37	21.45	21.64	23.41	23.05	23.46	24.12
MBSCS3	25	4	18.70	18.59	18.25	18.25	21.60	21.50	22.43	22.30	23.86	23.60	24.32	24.08
MBSCS4	20	5	20.40	20.00	19.56	19.09	23.30	22.85	22.84	22.47	24.58	24.71	24.29	24.36
MBSCS5	16.67	6	21.02	21.37	20.77	19.10	23.65	23.60	23.94	22.50	25.46	25.15	25.07	23.98
MBSCS6	12.5	8	21.58	22.41	21.65	20.26	24.01	24.15	24.60	22.87	25.15	25.34	25.26	24.12
MBSCS7	10	10	23.38	22.94	21.76	20.10	25.08	24.73	23.78	22.29	25.86	25.67	24.32	23.25
MBSCS8	8.33	12	23.26	22.88	22.40	20.91	24.76	24.28	24.16	22.39	25.34	24.94	24.65	23.00
MBSCS9	6.67	15	23.74	23.78	22.98	21.65	24.82	24.70	24.15	22.58	25.22	25.02	24.43	23.02
MBSCS10	5	20	23.63	23.21	21.30	19.51	24.11	23.74	21.76	20.10	24.29	23.92	21.89	20.24

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# Summary

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- There are much room for MBS
- Two-stage selection including MBS and PS can be more effective than single stage PS selection.
- CS and MBS can be combined to increase genetic gain.
- Even for traits with high heritability MBS can be more efficient than PS.
- MBS can be more efficient even if PGE is only low to moderate.



- The use of CS in main and elite breeding population can increase genetic gain (no time to present)



# Acknowledgment

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Dr Kevin Smith, Victoria DPI, Australia

Livestock and Meat Australia Ltd

Molecular Plant Breeding CRC