Experiment Objective : To study the feasibility of GP analysis by DNA isolated from Bulk of 1 x 4 Seeds in Hybrid Rice

Seeds of 2201 & 2233 were used as contaminants in bulk analysis of 2111 hybrid ,as both the hybrids are showing different reaction with "RM-164" marker than 2111 hence can be easily identified in bulk analysis.

DNA Isolation Protocol Followed:

Protocol -1:

1. Dehusked Rice seeds were soaked overnight in Petri dish containing DDH_20. Cut half portion seed contain embryo in 2 ml Microcentrifufe tube. **(For 4 seed bulking – 4 cut portions from 4 seeds were added in tube).**
2. Added one bead/tube and **500ul** Extraction Buffer, homogenized it in bead beater for 45 sec.
3. **Taken 1/4th of extract (125 ul) and make up the volume 500ul by adding extraction buffer.**
4. Incubated tubes at 65°C for 10 min.
5. Added 500 µL of "Chloroform: Iso-amyl alcohol (24:1)".
6. Centrifuged at "12,000 x *g"* for 10 min at room temperature.
7. Transferred the supernatant 300 ul to fresh 1.5 ml tube.
8. For precipitation of DNA, added equal volume of Iso-propanol and incubated at Room temperature .
9. Centrifuged at "12,000 x *g"* for 10 min at room temperature and discarded the supernatant.
10. Washed sequentially with 70% ethanol.
11. Centrifuged at "12,000 x *g"* for 5 min at RT.
12. Added 100% ethanol and centrifuged at room temperature for 5 min.
13. Decant ethanol and spine the pellet for 10 sec and removed residual ethanol.
14. The cleaned DNA pellet were air dried for 15 min and resuspended in 30 µL of low salt Tris-EDTA buffer. **(Please refer Samples no. 1 - 12, Gel Image-1)**

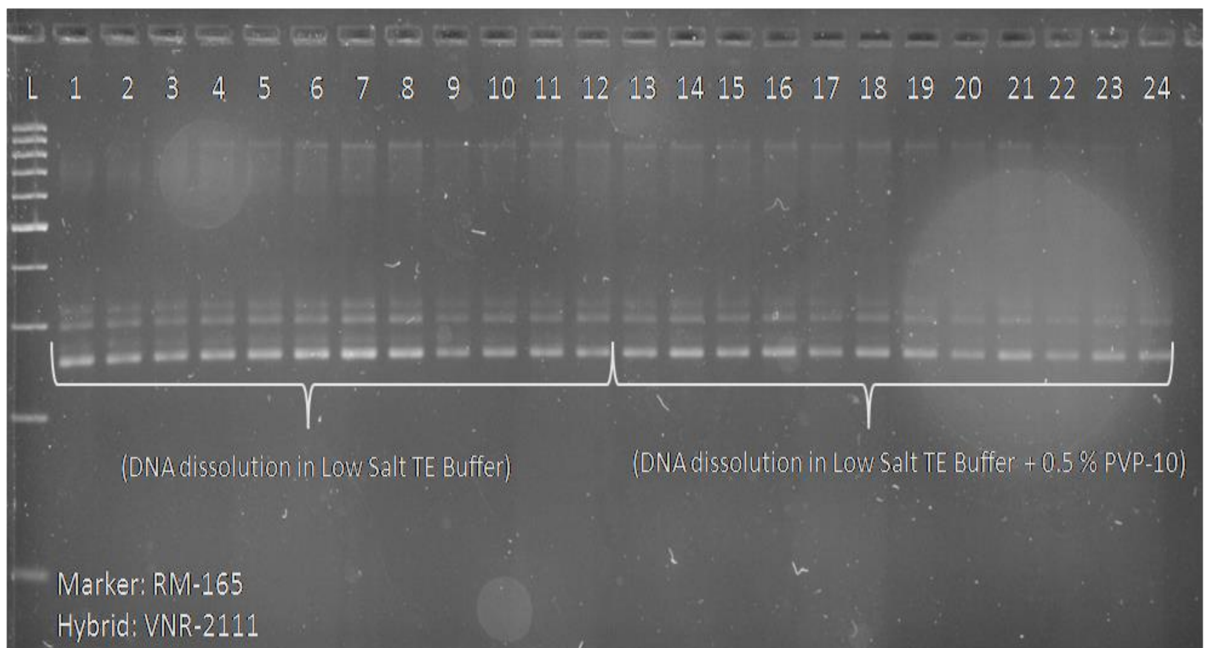
15. The cleaned DNA pellet were air dried for 15 min and resuspended in 30 µL of **low salt Tris-EDTA buffer with 0.5 % (w/v) Polyvinylpyrrolidone-10 (PVPP) (please refer samples no. 13 - 24, Gel Image-1)**

BUFFERS:

EXTRACTION BUFFER:		**100 ml**
0.1 M Tris_HCl pH 8.0	-	10 ml of 1 M
1.25 M NaCl	-	7.3 g
0.025 M EDTA pH 8.0	-	5.0 ml of 0.5 M
2% (w/v) CTAB	-	2 g
3% (w/v) Polyvinylpyrrolidone-40	-	3 g
RNase	-	100 ul/ 100 ml (Prior to used)

Results:

- In protocol 1, quality and quantity of DNA isolated from bulk of 4 X 1 half seed containing "Embryo" is good as it is evident from amplification of bands but band intensity is light (please refer Gel Image 1).)
- Gelimage-1

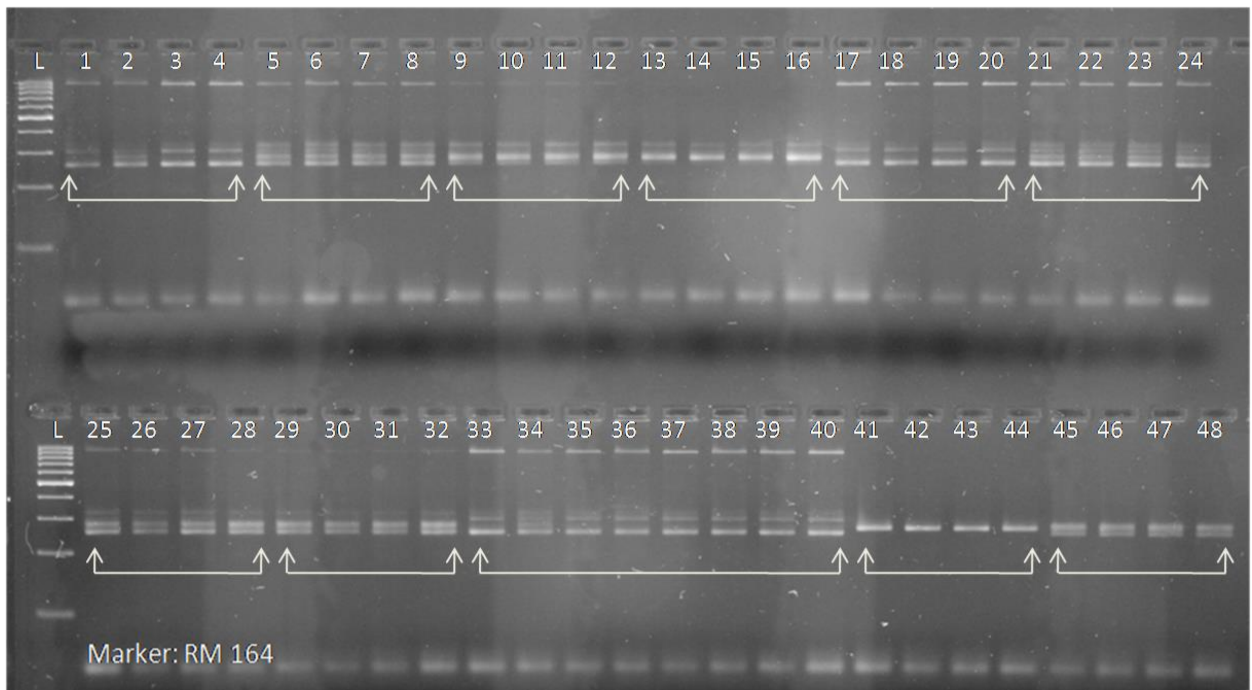


- After standardisation. 192 samples (bulk of 4 X 48) were analysed . The following combination of true to type and contaminants were used in analysis.

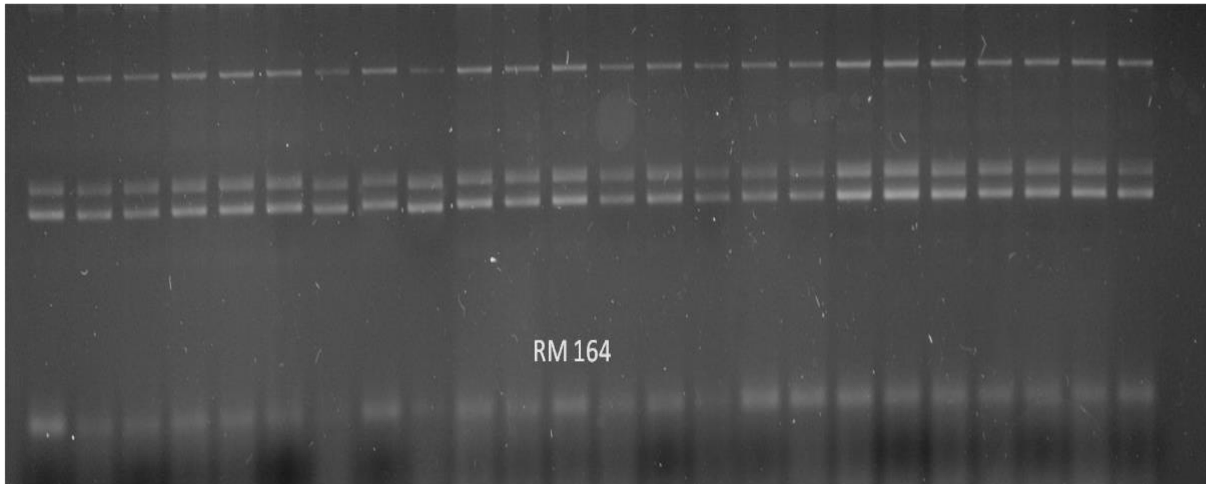
Bulk analysis of Rice seeds (1 x 4) -Gel Image - 2			
Sr. No.	Samples No.	Seeds Mixing Proportion	Ratio
1	1 to 4	VNR- 2111 : VNR 2201	4 : 0
2	5 to 8	VNR- 2111 : VNR 2201	3 : 1
3	9 to 12	VNR- 2111 : VNR 2201	2 : 2
4	13 to 16	VNR- 2111 : VNR 2201	1 : 3
5	17 to 20	VNR- 2111 : VNR 2233	4 : 0
6	21 to 24	VNR- 2111 : VNR 2233	3 : 1
7	25 to 28	VNR- 2111 : VNR 2233	2 : 2
8	29 to 32	VNR- 2111 : VNR 2233	1 : 3
9	33 to 40	VNR- 2111	4
10	41 to 44	VNR 2201	4
11	45 to 48	VNR 2233	4

If we look at image 2 intensity of bands is as good as we get it from seedling (Please refer Gel Image- 3), and contaminants can be easily identified , however the intensity of bands is changing with change in ratio.

Gel Image-2



Gel Image-3 DNA Isolated from Rice seedling



Conclusion: The above protocol can be used for Genetic Purity analysis in Rice by BULK method using seed as source of DNA.