Summary

1. Existing variation in anthocyanin-pigment distribution was analyzed in indica rice lines. These are selfed and made true breeding. The tested rice lines were classified into four classes based on the type and distribution of pigment in different tissues. Genotypes of these rice lines were predicted from their phenotypic analysis followed by known genetic nomenclature "C A P" system used for japonica subspecies. These genes are tentatively described as structural genes (C, A), regulatory genes (P, PI) and inhibitory genes (IP1-1 to IP1-6).

2. Specific PI alleles of the PI locus are mainly responsible for anthocyanin pigment distribution in rice. The class 1 lines (Purpleputtu and G 2237) contain PI1 allele as these are pigmented throughout the plant body including pericarp. The class II lines, carry either PI (R 27 P) or PI (G 962, TN 1013 and Crossa) or Pt (N 22W) allele as they show non-pigmented pericarp and pigmentation one or the other tissues. The class III, Whiteputtu and Hamsa being non-pigmented (acyanic) either carry null alleles of the regulatory genes or more likely recessive alleles of the structural loci. The class IV, N 22B showed brown pigmentation in pericarp (hence classified separately) and carries Pt allele.

3. The F1 progeny analyses of the crosses between Purpleputtu (purple), N 22 W (green) and N 22B (green) revealed the presence of the dominant inhibitor of leaf blade pigmentation, namely Ilb in N 22W and N 22B genotypes.

4. The F2 progeny of NW X PP and NB X PP segregated for leaf blade color in a dominant inhibitory interaction (13 green : 3 purple) with the following genotypes; Ilb Pl\W (9, green), Ilb Pl (3, green), ilb Pl\W (3, purple) and ilb Pt (1, green). The genotypes of Purpleputtu (ilb Pl\W), N 22W (Ilb Pt) and N 22B (ilb Pt) in terms of anthocyanin production were confirmed.

5. Based on spectrophotometry, thin layer chromatography and proton NMR spectroscopy of hydrolyzed methanolic extracts of rice tissues, the major and minor anthocyanidin pigments were determined as cyanidin and peonidin respectively. Further, the minor pigment, peonidin accumulates in significantly higher amounts in pericarp tissue.

6. Rice lines, G 962 and N 22B accumulating leucoanthocyanidins and proanthocyanidins respectively in pericarp tissues were identified. In these mutants, the
conversion of leucoanthocyanidin to anthocyanidin, mediated by anthocyanidin synthase, was blocked. It is concluded that the accumulation of proanthocyanidins is also due to a block at the conversion of leucoanthocyanidin to anthocyanidin. Most likely that the oxidative activity of anthocyanidin synthase (anthocyanidin synthase encode an NADPH dependent oxidoreductase) is blocked in the brown pericarped rice since the oxidation or oxidoreduction products of this reaction viz., phlobaphenes or cyanidin respectively were absent.

7. Effect of sunlight on anthocyanin biosynthesis in 15 rice genotypes was tested and accordingly classified them into three groups based on the extent of anthocyanin (red color) pigment accumulation in shoots of young seedlings. These are cyanic (accumulates copious amounts of anthocyanins), moderately cyanic (moderate amounts of anthocyanins) and acyanic (no anthocyanin synthesis).

8. The induction of anthocyanins in Purpleputtu was primarily mediated by sunlight as evident by the fact that the Purpleputtu seedlings grown for 5 days under cool fluorescent lights accumulated greatly reduced amounts of anthocyanins.

9. The 4d-old etiolated seedlings are most responsive (production of anthocyanins) to sunlight and a 30 min exposure to sunlight saturated the response which leads to a massive accumulation of anthocyanins with a peak at 24 h. The anthocyanins induced by sunlight are the same as those found in plants grown in continuous light.

10. The induction of anthocyanins in Purpleputtu seedlings is triggered by the UV-B component of SL. This conclusion was derived from the observation that the seedlings exposed to SL-filtered through window glass (WG), which cuts off UV-B component of the SL, RL, FR or BL completely lack anthocyanin pigments. Further, there is no induction of anthocyanins under any of the light treatments in Blackputtu.

11. The SL-mediated anthocyanin accumulation is modulated by RL and FR pulses. In rice, UV-B plays the primary role in the induction of anthocyanins and phytochrome modulates its response.

12. The UV-B responsive anthocyanin biosynthesis in rice is mediated through a specific phase of phenylalanine ammonia lyase activity. The SL mediated induction of PAL showed two peaks, peak-I at 4h and peak-II at 12h in shoots of Purpleputtu and only peak-I at 4h in shoots of Blackputtu seedlings. The other light treatments including WG, RL, BL, and FR indicated that only peak-I at 4h can be inducible in both the cultivars and is phytochrome dependent as evidenced by its reversible nature with
FR pulse. The peak-II at 12h in Purpleputtu is independent of FR pulse (not phytochrome dependent) and hence, UV-B light dependent. The photoinduction of peak-II of PAL and thereby accumulation of anthocyanin pigments depends exclusively on UV-B light. A correlation between the induction of peak-II of PAL activity and anthocyanin formation is also supported by the observation that peak-II is restricted to the cyanic Purpleputtu line.

13. In order to obtain cDNAs of early UV-B induced proteins as well as enzymes and regulatory protein factors involved in the anthocyanin pathway, a cDNA library was prepared from UV-B treated 4-d-old etiolated seedlings using λ-ZAP and XL1-Blue MRF' host system (Stratagene).

14. As a first step towards the characterization of the anthocyanin pathway genes in rice, the library was screened and the rice homologues of the Zea mays A, A2 and C flavonoid genes were isolated and partially characterized. These are: Os-DFR (1.5 Kbp), Os-ANS (1.6 Kbp) and Os-cMyb (1.4 Kbp) encoding anthocyanidin synthase, dihydroflavonol reductase and a regulatory protein factor respectively. The nucleotide sequence comparison between the rice cDNAs namely DFR, ANS and Os-cMyb and the corresponding Zea mays cDNAs revealed extensive homology (above 70%).

15. Southern and Northern analyses of anthocyanidin synthase gene were performed in rice. Genomic Southern analysis revealed that at least two sequences with extensive homology to Zm-A2 are present in the rice genome. The Ans gene specific transcript is inducible by UV-B light. The expression of anthocyanidin synthase gene is abundant in cyanic rice lines compared to acyanic genotypes. The presence of this transcript in various tissues of PP was demonstrated.

16. Differential expression pattern of the PAL and the DFR genes was observed in PP and N 22B seedlings. The PAL and DFR gene expressions are enhanced by UV-B in PP. The expression of the PAL gene transcript is totally absent in NB seedlings. The DFR transcript level was also affected in NB. The results revealed that the induction is genotype dependent and it is speculated that these changes are due to the Ilb allele.

17. The rice Actin 1 gene promoter-based transcriptional fusion expression constructs were developed for the molecular manipulation of anthocyanin pathway genes towards disease resistance in rice. These include both sense and antisense constructs for the almost entire set of the anthocyanin pathway genes the A, A2, Bz1, C, C2, R, P and PAL.