

Research Achievements of Crop Improvement

Germplasm exploration, conservation and maintenance

ICAR- Indian Institute of Soybean Research is active germplasm repository and has been acquiring and maintaining germplasm resources. Presently Institute has 4591 germplasm accessions with sources for many traits (Table 1):

Table 1: Germplasm accessions with unique characters available at ICAR-IISR Indore

Characters	Prominent Accessions
Photoperiod insensitivity	MACS 330, EC 325097, EC 333897, EC 34101, EC 325118, EC 390977 and EC 483049
Long Juvenility	AGS 25
Drought Tolerance	EC 538828, PI 416937, Young, EC 602288
Disease resistance	
Rust	EC 241778 and 241780
Yellow Mosaic Virus	UPSM 534, PLSO 84, EC24660, B463
Rhizoctonia Root Rot	AGS-48, EC 325099, EC 34117
Insect resistance	
Girdle beetle	TGX 863-26E, SREC 56A, TGX 302-2A, TGX 849-D
Defoliators	EC 333902, VP 1165
High oil Content	AGS 191, NRC 7, G76 (22-23%)
High oleic acid	IC 210 & NRC 106 (42%) low linolenic acid: VLS 59 (4%)
Null trypsin inhibitor:	NRC 101, NRC 102
High protein:	G 288, G 688 (44%)

Development of high yielding varieties

ICAR-IISR has co-ordinated in the development of 102 soybean varieties in which 30 varieties are in seed chain. Among these varieties ICAR-IISR itself has developed five soybean varieties (NRC 2, NRC 12, NRC 7, NRC 37, NRC 86) and one more soybean variety (NRC 77) is under the process of notification. Three varieties NRC 7, NRC 37 and NRC 86 are under seed chain (Fig 1).



NRC 7



NRC 37



Fig 1: Soybean varieties developed at ICAR-IISR which are currently under seed chain.

Breeding material developed at ICAR-IISR is shared with AICRP centres and variety like JS 97-52 has been developed by selection at Jabalpur. Varieties under seed chain have been protected under Protection of Plant Varieties and Farmer's Right Act-2001. Varieties with useful characters have been summarized in Table 2:

Table 2: List of soybean varieties with useful traits

Characters	Prominent varieties
High yield potential	JS 97-52, JS 95-60, NRC 37, JS 93-05, NRC 7, JS 335
Early Maturity	JS 95-60, JS 93-05, LSb 1, NRC 7, JS 20-34
Tolerance to drought	NRC 7, Hardee, JS 71-05
Multiple disease resistance	VLS 63, Pusa 9814, PS 1347
Resistance to YMV	JS 97-52, SL 688, SL Pusa 9814, SL 525, PS 1347
Tolerance to insect pests	TAMS 978-21, Pratap Soya 1 and 2,
High seed longevity	JS 335, MAUS 61-2, JS 93-05, NRC 37, JS 97-52
Improved oil quality (Low linolenic acid)	VLS 59

Molecular characterization of germplasm has identified five accessions (PI 175192, EC 333915, CF 492, EPS 71 and DT 21) for the presence of unique SSR alleles. An advanced backcross mapping population (BC2F4) has been developed from interspecific cross JS 335 x *Glycine soja* and QTLs has been identified for 100 seed weight, number of pods and seed yield per plant.

Development of specialty soybean

Institute validated the molecular markers for this trait. Using this information a co-ordinated DBT funded project was taken up to convert JS 97-52, DS 97-12, JS 93-05 and MACS 57 to their null-KTI version in which IARI, New Delhi and ARI, Pune were involved as co-operating centres. With further identification of source of null-lox II and development of NRC 109 as null-Lox II, Institute has initiated another DBT funded project involving these centres to convert these varieties into null version of both of the traits.

Soybean genotypes IC 210 and NRC 106 have been identified as source of high oleic acid and commercialized. For further increasing the oleic acid content, these lines have been intercrossed and crossed with other relatively higher oleic acid containing germplasm lines and varieties and transgressive segregants with >60% oleic acid content have been identified.

Other specialty soybean like vegetable soybean (NRC 105) and high oil (25%) have been identified.

Conventional and molecular techniques for development of drought tolerant varieties and lines

Institute developed a drought tolerant, bold seeded and high oil containing variety NRC 7. Another drought tolerant variety JS 97-52 is a selection from the breeding material of ICAR-IISS and is terminal drought tolerant material. Using these varieties and other drought tolerant lines like EC 538828, PI 416937 and Young, Institute has developed 386F₄, 122F₅, 174F₆ and 46F₇ lines which are being tested in drought nursery.

Genomic approaches have been applied for identification of genomic regions responsible for drought tolerance. Differential display RT-PCR technique has identified two drought responsive transcripts from the drought tolerant cultivar NRC 7 under water deficit conditions. Expression profiling of soybean conserved miRNAs obtained from the roots and leaves of Indian soybean cultivars JS 335 (susceptible) & NRC 7 (resistant) identified small RNA biomarkers associated with drought tolerance. Soybean genome data has been searched for identification of genes associated with drought tolerant. Total 3 gene families have been identified and are being analyzed. RNAi and VIGS approach is being used to identify genes related to drought tolerance. Construction of vectors is under progress.

Breeding for developing photoinensitive and long juvenile soybean varieties

Seven photoinensitive lines (MACS 330, EC 325097, EC 333897, EC 34101, EC 325097, EC 390977 and EC 483049) have been identified by screening 2100 germplasm lines under extended day length created by using incandescent bulbs. These lines have been characterized for photoperiodic (*E1*, *E3*, *E4*) and maturity (*E2*) genes and used for deciphering the genotype of major soybean cultivars. Soybean varieties have also been characterized for growth habit genes and it was found that most of them are determinate.

In order to develop soybean with buffering for delayed sowing condition a long juvenile line AGS 25 has been identified (Fig 2). This line delays flowering and is capable of good growth under short day conditions. Other suitable variety identified is JS 97-52. Although NRC 37 also takes up good growth under delayed sown conditions yet reproductive phase is greatly affected. AGS 25 and JS 97-52 have been hybridized with a number of conventional juvenile

lines and advanced breeding material with buffering for delayed sowing condition is being identified.

AGS 25



JS 97-52



NRC 37



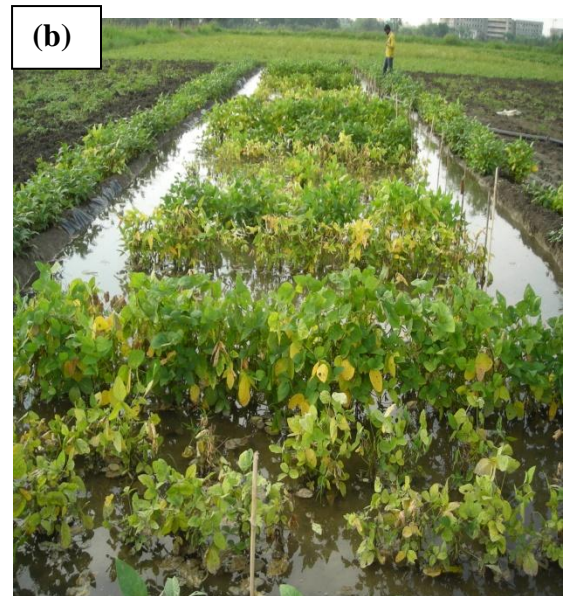
Fig 2 Identification of suitable donors for long juvenility trait under delayed sowing conditions.

Understanding the physiological basis of water logging tolerance

Water logging tolerance screening techniques for flowering and early vegetative stages have been standardized. Screening of soybean germplasm for water logging tolerance has identified JS 97-52 as the highly tolerant variety. This variety has been compared with susceptible varieties and two features (1) quick appearance of adventitious roots on water logging (Fig 3) and (2) Presence of more and large aerenchyma were attributed to its high level of tolerance. Development of recombinant inbred lines for identification of major QTLs is in progress.



(A) Field condition after 1 week after inundation



(B) Field condition after 15 days after continuous inundation



(c)

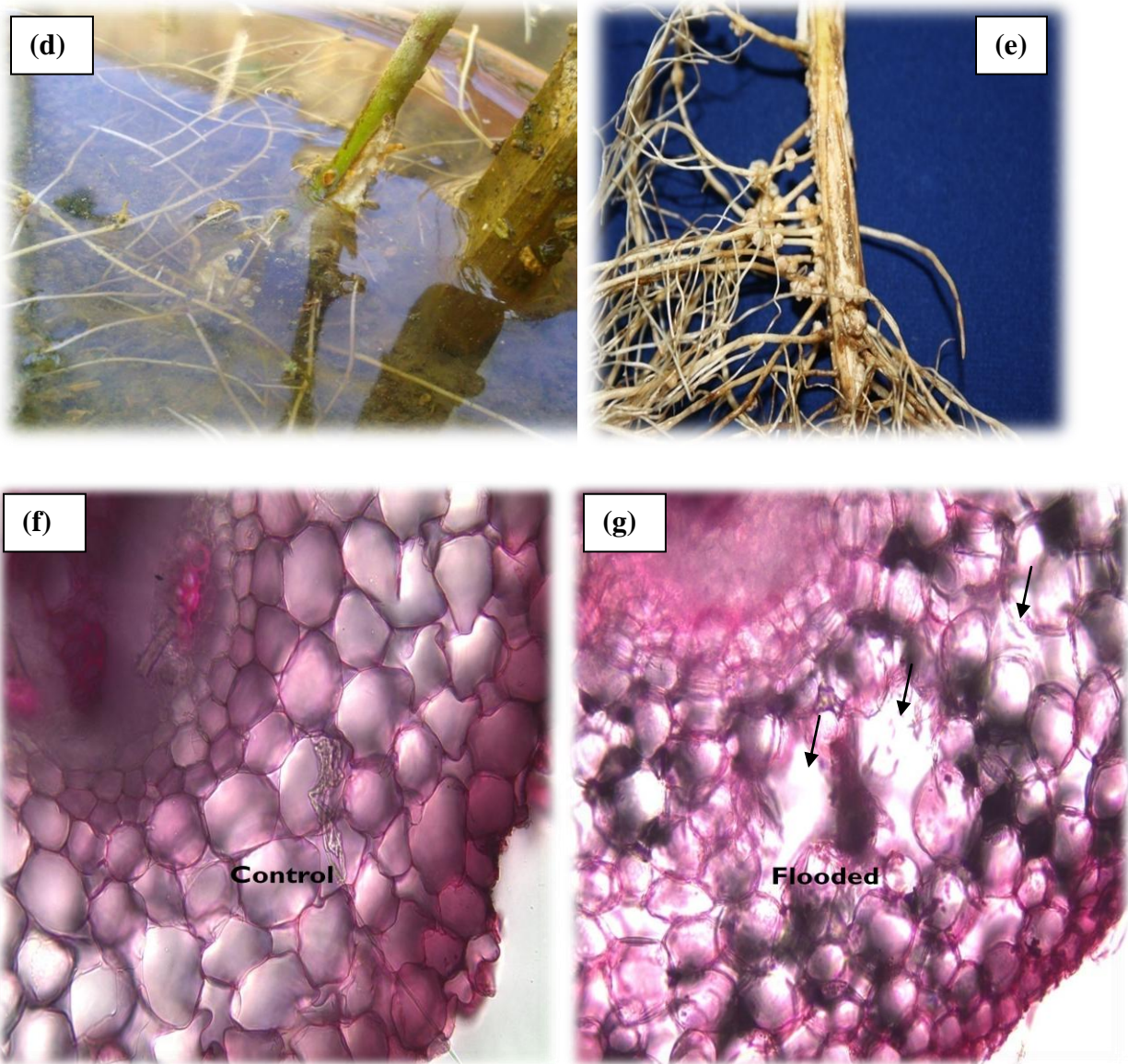


Fig 3: Development of field screening technique for water logging tolerance (a-c) Emergence of adventitious roots during water logging conditions in JS 97-52 (d & e) Presence of aerenchyma (arrows) under water logging conditions in JS 97-52 (f & g)

Development of plant regeneration and genetic transformation protocols

Institute has developed efficient plant regeneration protocol using half seed, embryonic axes and cotyledonary nodes as explants (Fig 4). Also standardized *Agrobacterium* mediated genetic transformation protocol using half seed explants.

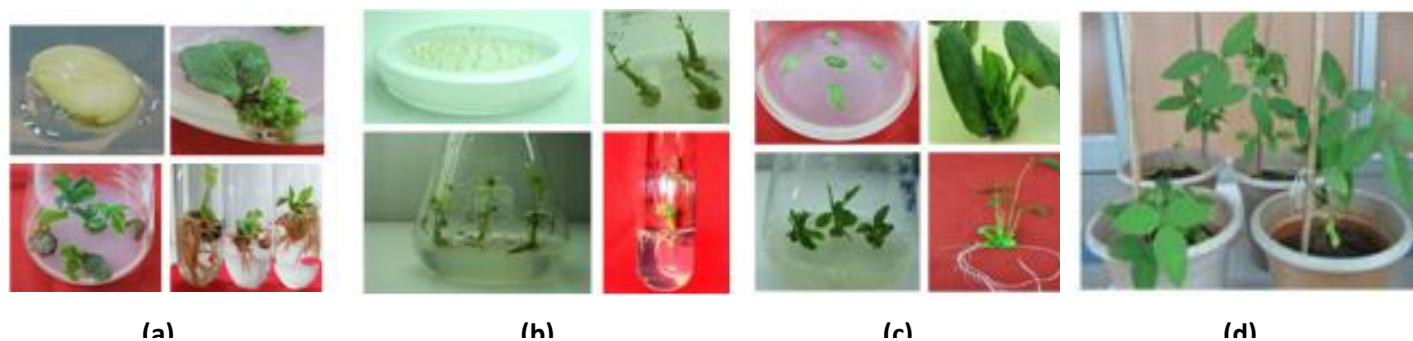


Fig 4: Development of plant regeneration protocol from (a) half seed (b) embryonic axis (c) cotyledonary nodes and (d) hardening of regenerated plants.

Conventional and biotechnical approaches for YMV tolerance

Two sources (UPSM 534 and *Glycine soja*) for YMV tolerance are available in India. UPSM 534 has been widely used in development of YMV tolerant soybean varieties in Northern Zone where YMV causes severe yield losses. However, rapid spread of YMV in central India has prompted us to use UPSM 534 derived resistance in JS 335. In collaboration with PAU, Ludhiana (hot spot for YMV) JS 335 is in advanced stage of conversion to YMV tolerant one.

G. soja, a source of YMV tolerance, has not been used in breeding programmes due to transfer of undesirable characters along with the tolerance. Institute has initiated backcrossing programme and introgressed YMV tolerance from *G. soja* to JS 335. Institute has also identified molecular markers for this trait for MAS.

Complete genome of Begomovirus infecting soybean in central India has been characterized and PCR based assay has been developed to detect major begomoviruses (Family: Geminiviridae) infecting soybean India. Agro-infectious binary plasmids harbouring head to tail tandem repeats of Mungbean yellow mosaic India virus-sb genome have been developed to screen the soybean genotypes for resistance against Yellow mosaic disease (YMD) under controlled conditions (Fig 5 A & B).



Fig 5 A: Vector control of agroinfection



Fig 5 B: Agroinfection with developed vector

RNA silencing based binary plasmids to repress Mungbean yellow mosaic India virus-sb derived genes were developed and their silencing efficiency was validated through gene silencing assays in YMD susceptible cultivar JS335 (Fig 6 A and B).



Fig 6 A: JS 335 plant with YMV infection



Fig 6 B: JS 335 plant after gene silencing

Soybean microRNAs (miRNAs) that display propensity to repress begomovirus derived ORFs and genomes have been identified

Conventional and molecular approaches for rust resistance

Two germplasm accessions (EC 241778 and EC 241780) have been identified for rust resistance and varieties like DSb 21, DSb 23-2, KDS 344 and KDS 726 have been developed by Dharwad and Kasbe digraj centres of AICRP through marker assisted selection. For rapid conversion of material and for phenotyping independent breeding Institute has undertaken the

programme to identify molecular markers for rust resistance. In this programme through hybridization EC 241780 and JS 335 it was found that rust resistance is imparted by single recessive gene. Phenotyping of F_{2:3} and cosegregation studies would identify molecular markers for this trait.

Whole genome resequencing has been taken up for comparative genomic studies between reference genome Williams and rust resistant line EC 241780 for identification of allelic variations between NBS-LRR regions of two genomes.

Identification of soybean genotypes for nutrient uptake efficiency

Soybean varieties and genotypes have been screened for phosphorus uptake and nitrogen fixation efficiencies. Root length, root diameter, root surface area have been identified as main trait that contribute to improved phosphorus uptake. Soybean varieties JS 335, JS 80-21, MAUS 81, NRC 37, MAUS 61-2, MAUS 71 and Gaurav were identified as improved phosphorus efficient varieties. JS 97-52, PS 1225 and PS 1029 have been identified as the best varieties for nitrogen fixation efficiency. Varieties like Hardee, NRC 86, MACS 57, NRC 86, JS 20-34 and ADT-1 are other good varieties.

Development of mapping populations and identification of QTLs

Nested association mapping population (NAM), Multiparent Advanced Genetic Intercross (MAGIC) population and advanced backcross populations are in advanced stage of development. QTLs have been identified for 100 seed weight, number of pods and seed yield per plant. Introgression lines for transfer of YMV resistance from *G. soja* have been developed.

Seed technological interventions for better crop growth and yield

Institute has found that thin layer polymer seed coating (Fig 7) with fungicides Carboxin + Thiram (2 + 1 g/kg seed) or Carbendazime + Thiram (2 + 1g/kg seed) along with Thiomethoxam (3 g/kg seed), is more effective than conventional dry dressing in providing protection to the crop from the beginning of growth.

Recommended biocontrol agent (*Trichoderma sp.*) has been found to grow endophytically in 100 percent soybean plant, when applied through seed polymer coating and found effective to control fungal diseases.

Seed treatment with Salicylic acid @75mg/kg of seed and foliar application @100ppm at vegetative and pod filling stage was effective to control diseases and to improve seed yield and quality.

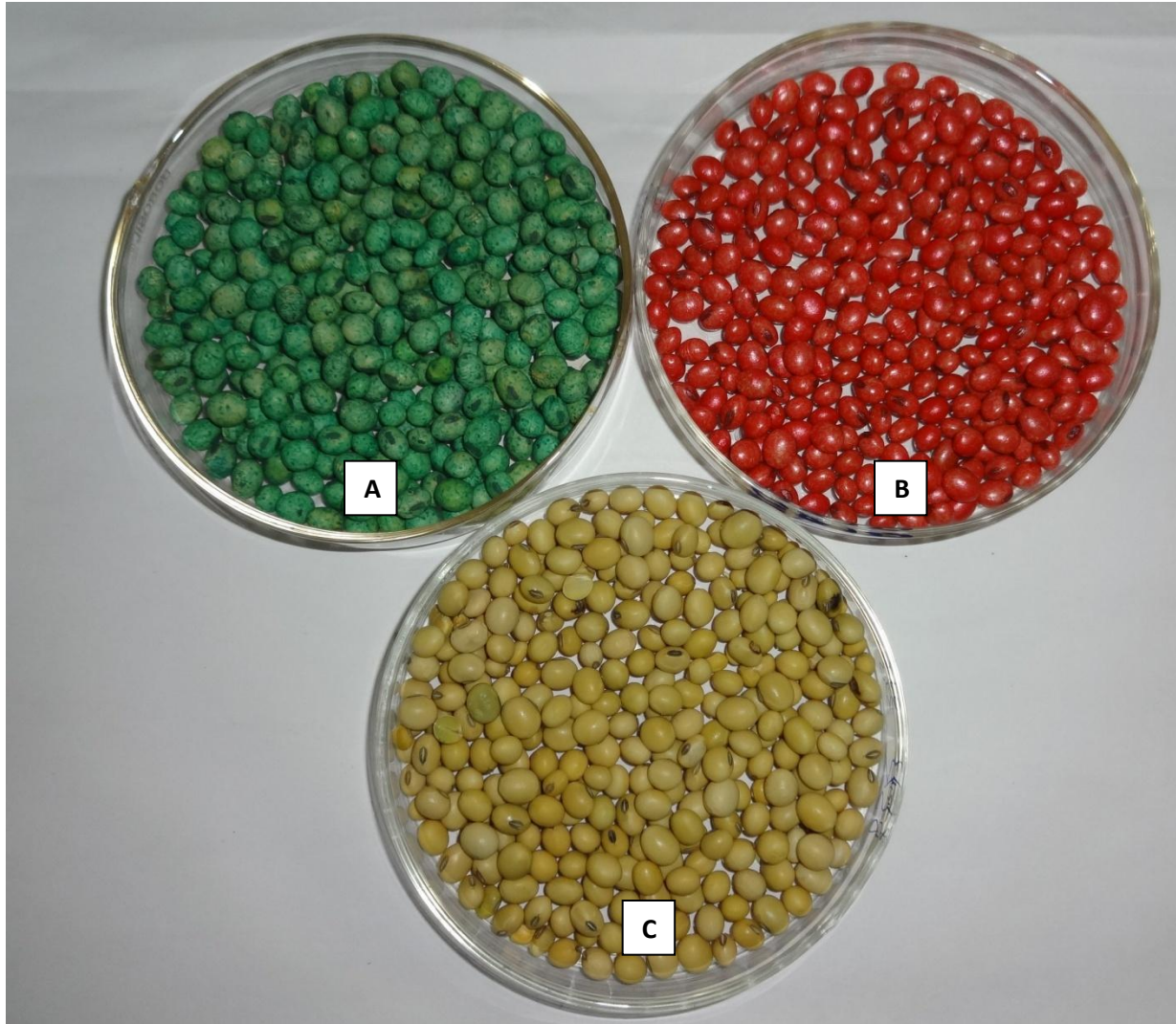


Fig 7: Thin layer polymer coated (A & B) and uncoated (C) soybean seed.