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PANCHAGAVYA FOR SUSTAINABLE AGRICULTURE

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Abstract

Panchagavya is a mixture used in traditional Hindu rituals that is prepared by mixing five ingredients. The three direct constituents are cow dung, urine, and milk; the two derived products are curd and ghee. It has the potential to play the role of promoting growth and providing immunity in plant system. Panchagavya contains macro and micro nutrients and play a significant role in growth and development of plants. It comprises of different amino acids, nutrients, increment controllers like Auxins, Gibberellins and furthermore valuable microorganisms like pseudomonas, Azotobacter and phosphate Solubilizing microbes and so forth Panchagavya additionally contains plant development substances, for example, IAA and Gibberellic acid, just as other fundamental plant supplements. Because of synthetic fertilizers contamination and unfavourable impact on the dirt wellbeing, Panchagavya plays a significant role in organic farming as a substitution for synthetic fertilizers. Besides, considers uncovered the presence of a few useful microorganisms like Actinomycetes, Pseudomonas, photosynthetic microscopic organisms and parasites in Panchagavya which assumes a significant part as development enhancer for harvests and make soil greater efficiency.

Introduction:

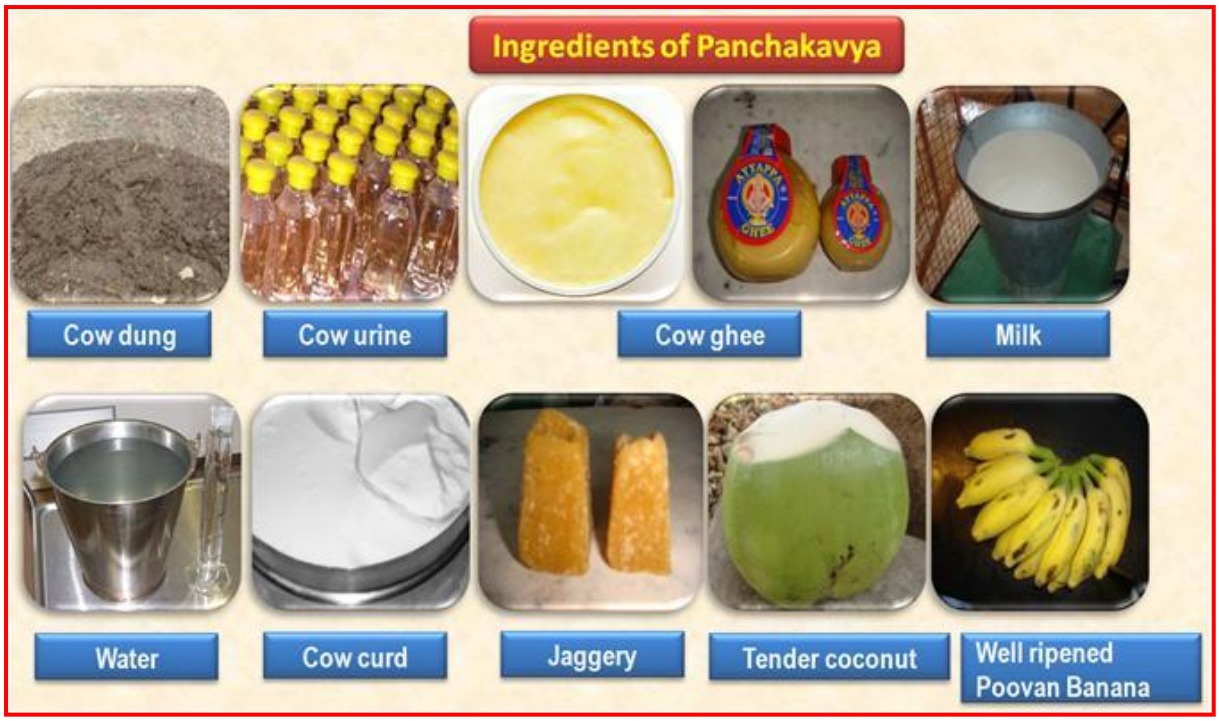
Organic agriculture is a production management system that encourages and improves agro-ecosystem sustainability; including bio-diversity, soil biological activity, and biological cycles. It emphasizes the use of management practices, especially off-farm inputs, in light of the fact that regional conditions necessitate locally tailored systems. Panchagavya is an organic form that in Sanskrit refers to a mixture of five products made from cow milk, ghee, curd, dung, and urine (all of these products are referred to as "Gavya" individually and as "Panchagavya" collectively). In the Vedas (divine texts of Indian wisdom) and Vrikshayurveda, Panchagavya is listed and its uses has been elaborated (Natarajan, 2002). In recent years, the usage of Panchagavya in organic farming has gained in popularity in India, in most of the states Organic farming has been gaining more attention in recent years as a result of the detrimental effects of chemical inputs. The United Nations' Food and Agricultural Organization (FAO) reported that organic agriculture covered 37.2 million hectares in 2011, three times more than in 1999. Synthetic pesticides, inorganic fertilizers, genetically modified organisms, and other chemicals are strictly not allowed to use in organic farming. Bio fertilizers or conventional organic formulations made from organic materials/waste are also recommended for plant nutrition (Badgley *et al.*, 2007). Organic farming relies heavily on Panchagavya. It is described in Hindu Vedas such as the Vrikshayurveda as a source of nutrients for the soil (Natarajan, 2002). Effective Micro

Organisms (EMO) in Panchagavya were a combine culture of naturally produced, useful microbes that enhanced soil fertility, primarily lactic acid bacteria (*Lactobacillus*), yeast (*Saccharomyces*), actinomyces (*Streptomyces*), photosynthetic bacteria (*Rhodopsuedomonas*), and certain fungi (*Aspergillus*) (Natarajan, 2002). Panchagavya affects the quality of fruits and vegetables significantly. It is used in various ways like as seed treatment, foliar spray, and soil application along with irrigation. Panchagavya is a sustainable approach in agriculture used by farmers in South India. Synthetic fertilizers and pesticides have wreaked havoc on the environment, so organic fertilizers and pesticides have emerged as a viable alternative. Panchagavya is also known to enhance crop growth and establishment.

Preparation methodology

Panchagavya is a special preparation made from five by-products of cow along with certain other ingredients, has the potential to play the role of promoting growth and providing immunity in plant system. Panchagavya plays a major role in organic farming. Ingredients used for preparation of Panchagavya.

1. Fresh cow dung 5 kg
2. Fresh cow's urine 3 lit.
3. Cow's milk 2 lit.
4. Cow's curd 2 lit.
5. Cow's ghee 500 gm
6. Jaggery 500 gm
7. Sugarcane juice 3 lit.
8. Ripe banana fruit 1 dozen
9. Tender coconut water 3 lit.



Panchagavya can be prepared as follows:

In a wide mouthed plastic drum, put five litres of cow dung slurry, three litres of cow's urine, two litres of cow's milk, two litres if curd prepared from cow's milk and one litre of cow's ghee. Mix these ingredients thoroughly and the mouth of the vessel must be covered with a clean cloth for ensuring aeration for the fermenting unit. The vessel with contents must be placed in a shady place. The ingredients must be mixed thoroughly with hand every day in the morning and evening for 10-15 days. It can be stored and used for the next 30 days. Before spraying on plants, three litres of Panchagavya may be mixed with 100 litres of water. Since ghee does not dissolve easily, power sprayer is the best option for spraying.

Beneficial Effects of Panchagavya:

Panchagavya is a component of crop production and it plays a crucial role in each and every component of crop management like integrated soil fertility management, integrated pest management, and integrated disease management.

Effect of Panchagavya on soil fertility and productivity

1. Panchagavya improves fertility status in soils by increasing macronutrients, micronutrients and beneficial microorganisms thus increase soil health.
2. It improves water holding capacity of soils because it acts as organic manure.
3. It encourages growth and reproduction of beneficial soil microorganisms
4. Increases nutrient uptake in plants and enhances plant growth.

Use of Panchagavya as growth promoter

In jasmine, spraying two rounds of Panchagavya, one before the flower initiation and another during bud setting phase ensured continuous flowering. In annual mooring spraying doubled the stick yield besides giving resistance to pests and diseases (Vivekananda 1999) The current trends in organic practices showed improved yields in crops of rained areas in India, especially during drought years (Singh *et al*. 2001; Ramesh *et al.* 2005) [5]. Studies have shown increased yields where the farmer has used organic practices (Singh *et al*. 2001; Ramesh *et al*. 2005) [5] in crops like chilli (Subhashini *et al*. 2001), moringa (Beaulah 2001) [12], green gram (Somasundaram *et al*. 2003) and french bean (Selvaraj 2003). It can be concluded that Panchagavya as an organic growth-promoter for small and marginal vegetable growers (Boomathi 2006). The cost-benefit to farmers was greatest when Panchagavya was used as agrowth promoter and proved as the cheapest, while AmritPani, and Bokashi were the costliest alternative input (Francis & Smith 2006).

Disease and pest control

(Boomiraj *et al.,* 2004) revealed that Panchagavya was powerful against leaf miner (Amsacta biguttula) and white fly (Bemisia tabacci) in bhendi. Comparative outcomes were seen by (Mudigora *et al.,* 2009) in cabbage and sorghum. Cow dung is viable excrement for diminishing the bacterial and parasitic pathogenic infections. It showed positive reaction in suppression of

mycelial development of plant pathogenic parasites like Fusarium solani, F.oxysporum and Sclerotinia sclerotiorum (Basak and Lee, 2002).

Effect of Panchagavya on plants

Leaf

Plants sprayed with Panchagavya invariably produce bigger leaves and develop denser canopy. The photosynthetic system is activated for enhanced biological efficiency, enabling synthesis of maximum metabolites and photosynthetic.

Stem

The trunk produces side shoots, which are sturdy and capable of carrying maximum fruits to maturity. Branching is comparatively high.

Roots

The rooting is profuse and dense. Further they remain fresh for a long time. The roots spread and grow into deeper layers were also observed. All such roots help maximum intake of nutrients and water.

Advantages of Panchagavya

- It improves soil health and fertility
- It is used against pest and diseases
- It increases yield and quality of produce
- No chemicals are used
- Eco-friendly approach
- Cost required for preparation is less
- No special techniques is required
- It gives multiple uses
- Reduces cost of cultivation by reducing chemicals like Fertilizers, pesticides,
- Fungicides, growth regulators etc
- Farmer friendly method

Panchagavya application methods

1. **Spray system**:

3% solution was found to be most effective compared to the higher and lower concentrations investigated. Three litres of Panchagavya to every 100 litres of water is ideal for all crops. The power sprayers of 10 litres capacity may need 300 ml/tank. When sprayed with power sprayer, sediments are to be filtered and when sprayed with hand operated sprayers, the nozzle with higher pore size has to be used.

2. **Seed/seedling treatment**

3% solution of Panchagavya can be used to soak the seeds or dip the seedlings before planting. Soaking for 20 minutes is sufficient. Rhizomes of Turmeric, Ginger and sets of Sugarcane can be soaked for 30 minutes before planting.

3. **Flow system**

The solution of Panchagavya can be mixed with irrigation water at 50 litres per hectare either through drip irrigation or flow irrigation

4. **Storage of seeds**: In this process, 3% of Panchagavya solution should be used to dip the seeds before drying and storing them for long shelf life, vigour and more percentage of germination.

Periodicity of Spraying

- **Pre-flowering stage:** Spray Panchagavya once in 2 weeks, two sprays depending upon duration of crops.
- **Flowering and Pod development stage:** Spray Panchagavya once in 9 to 10 days. Generally 2 sprays are sufficient
- **Fruit / Pod maturation stage:** Spray Panchagavya once during pod maturation phase.

Conclusion

The increasing concern for environmental safety and global demand for pesticide residue free food has evoked keen interest in crop production using eco-friendly products which are easily biodegradable and do not leave any harmful toxic residues besides conserving nature. So it is necessary to use natural products like Panchagavya to produce chemical residue free food crops and hence Panchagavya can play a major role in organic farming.

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PROCEDURE FOR GRAIN P ANALYSIS

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Phosphorus (P) is a macro nutrient and a component of nucleic acids, plays a critical in the metabolism of plants, playing a role in the transfer and storage of energy from photosynthesis and the metabolism of carbohydrates, of which grain production is an important result. It is also a structural component of the nucleic acids of genes and chromosomes and of many coenzymes, phosphoproteins and phospholipids. Application of P is recommending as a major agronomical practice for increasing crop yield, given the fact that about 5.7 billion hectares worldwide lack sufficient available P (Batjes 1997). P is a non-renewable resource and some commercially viable sources of rock phosphate are likely to be exhausted in the next century (Lott et al. 2011). Thus, strategies including breeding P efficient cultivars, optimizing P fertilizer rates, and minimizing soil P mining had been proposed for better P management of cropping systems (Ismail et al. 2007).

A large fraction of the P taken up by plants during vegetative growth is translocated to the developing seed. The phosphorus stored in mature seeds of grain and legume crops is estimated to represent a sum equivalent to more than 50 % of phosphate fertilizer applied annually worldwide (Lott et al. 2000). Consequently, seed phosphorus has value as a target in P management. Typically, inorganic P (Pi) consists of ~10 % of total phosphorus in cereal grains, whereas the majority of grain phosphorus, accounting for 50– 80 %, occurs in the form of phytic acid (PA), a myoinositol hexakisphosphate (Raboy 2007). The potential negative charges on the phosphate groups of PA bind various cations, especially K + and Mg2+, to form a salt called phytate (Raboy 2007). Pi is of higher bioavailability for humans and animals. By contrast, PA-P is indigestible for humans and non-ruminant animals such as poultry, swine, and fish, because they lack the enzyme to decompose phytic acid. In addition,

Phytic acid also forms complexes with proteins, digestive enzymes and minerals, and as such is considered to be an anti-nutritional factor (Lia et al. 2008). Substantial efforts have been made to identify mutants with impaired phytate biosynthesis, with low PA mutants being available now for key staple food crops such as barley, rice, and wheat. This development might offer potential benefits for the phosphorus nutrition of humans and animals (Raboy 2009).

Phospshorous deficiency leads to poor appetite, anemia, muscle weakness, bone pain, bone disease (osteomalacia, rickets), confusion, increased susceptibility to infections. But if your phosphate levels are too high, it can remove calcium from your bones, which makes them brittle. It can also cause calcium deposits in your eyes, lungs, heart and blood vessels, which

increase your risk of heart attack, stroke and death over time. Phosphate tests are very useful for measuring phosphate levels in people with malnutrition (where their diet doesn't contain the right amount of nutrients to meet their body's demands). A phosphate test can also be used to check for a condition called ketoacidosis, which sometimes affects people with diabetes.

Many analytical techniques have been employed to characterize forms of phosphorus in plant material. Among them, spectrophotometric methods based on molybdenum blue colouration are still the most widely used. However, colourimetric methods may not be sufficient to characterize different species of organic and inorganic P. To respond to these challenges, in recent years, many spectroscopic, chromatographic, and electrophoretic techniques have been developed and successfully used by workers (Jinbong et al., 2015).

Detailed information of rice grain grain phosphorous analysis by Vanadomolybdate yellow colour method using a spectrophotometer (Varian CARY 50) (Piper 1966).

1. Take 0.5g/1g of seed into borosil 50ml test tube
2. Add 5ml/10ml of tri acid (triacid acid (nitric acid (ISOCHEM): sulphuric acid (ISOCHEM): perchloric acid (SDFCL) of 3:2:1 ratio) into the tube respectively and put in the digestion unit until color changes to white liquid
3. Wait until tubes get cooled, after removal of tubes from digestion unit and completely transfer the tri acid solution into 50ml volumetric flask by filtering
4. After filtering the solution, take 5ml of tri acid liquid into 25ml of volumetric flask and add 5ml of Barton's reagent and make up to the volume with distilled water and wait for 15-30 minutes and take readings in spectrophometer at 420nm after adjusting the transmittance of the meter to 100 with a blank.
5. The concentration of phosphorus in the solution was deduced from the standard curve from which the percentage of phosphorus content of the sample was calculated

$$\text{P content on moisture basis } = \frac{X}{10^6} \times 25 \times \frac{V}{5} \times \frac{100}{W} \times \frac{100}{100 - M}$$

Where X = corresponding ppm from standard graph; V = volume of tri-acid extract; W = weight of the sample taken; M = moisture content of the plant sample

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SYSTEM OF RICE INTENSIFICATION

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The System for Rice Intensification (S.R.I) was developed by French Priest Father Henri de Laulani in Madagascar in the 1980"s in an effort to find sustainable agricultural practices which lead to higher productivity, optimum use of capital and labour, less input cost and less requirement of water (Zotoglo, 2011). SRI, as opposed to traditional rice production, involves alternate wetting and drying of rice fields (Kepha, Bancy and Patrick, 2014). In the broadcasting, one has to use a minimum of 100kg of rice seeds for one hectare, in planting one required about 30-60kg of seeds or so. But in SRI, only 4-10kg of seeds are required for one hectare (Randriamiharisoh, R, et al (2006). Therefore, this reduces input cost for the farmers and also less drudgery.

Principles of system of rice intensification (SRI): Laulanie established the following six key elements of SRI (Uphoff, 2007). The key physiological principle of SRI practices is to provide optimal growing conditions to individual rice plants so that tillering is maximized and phyllochrons are shortened, which is believed to accelerate growth rates (Nemoto et al., 1995). There are six principles guiding system of rice intensification (SRI), these are:

1. Seedlings get transplanted at a much younger age;
2. Only single seedling, instead of a handful of seedlings get planted in each hole;
3. Increased use of organic fertilizer to enhance soil fertility;
4. Intermittent water application to increase wet and dry soil conditions, instead of continuous flood irrigation;
5. Plants are spaced wider apart instead of close, dense planting, with seed rates of 50-100 kg/ha, plants were set out carefully and gently in a square pattern, 25x25cm or wider if the soil is very good; the seed rate is reduced by 80-90%, netting farmers as much as 90 - 95 kg of rice per hectare and
6. Rotary weeding to control weeds and promote soil aeration.

In SRI paddy cultivation, less quantity of seeds - 2 kg / acre is required. Hence fewer plants per unit area (25 x 25 cm), 16plants per square meter, one seedling per hill and 64000 plants per acre, whereas in mainstream chemical intensive paddy cultivation requires 20 kg seed per acre.

Nursery Management:

- Seed rate 2 kg/acre
- Nursery area 1 cent / acre
- Select healthy seeds
- Pre-sprouted seeds are sown on raised nursery bed

- Prepare nursery bed like the one prepared for garden crops
- Apply a layer of fine manure
- Spread sprouted seed sparsely
- Cover with another layer of manure
- Mulch with paddy straw
- Water carefully

Main field preparation

- Land preparation is not different from regular irrigated rice cultivation.
- Levelling should be done carefully so that water can be applied very evenly.
- Provide a canal at every 3m distance to facilitate drainage.
- Draw lines both ways at 25x25cm apart with the help of a marker and transplant at the intersection.

Transplanting

- 8-12 days old seedlings are transplanted
- Care should be taken during pulling out and transplanting of seedlings
- A metal sheet is inserted 4-5 inches below the seed bed and the seedlings along with soil lifted without any disturbance to the root.
- Seedlings are transplanted shallow and therefore establish quickly. Single seedling with seed and soil are transplanted by using index finger and thumb and gently placing them at the intersection of markings.
- Initially requires 10-15 persons to transplant one acre.

Irrigation and water management

- The purpose of irrigation is just to wet the soil, just enough to saturate the soil with moisture
- Subsequent irrigation is only when soil develops fine cracks.
- Regular wetting and drying of soil results in increased microbial activity in the soil and easy availability of nutrients to the plants.

Weed management

- Absence of standing water leads to more weed growth in SRI.
- Incorporate the weeds in the soil by moving the weeder between the rows
- Weeds close to the hills/tillers have to be removed by hand

SRI encourages rice plant to grow healthy with:

- Large root volume
- Profuse and strong tillers - Maximum tillering (30 tillers/plant can be easily achieved; 50 tillers per plant are quite attainable) occurs concurrently with panicle initiation. Under

excellent management even 100 fertile tillers per plant or even more can be achieved due to early transplanting and absence of die back of roots.

- Non lodging
- Big panicles
- More and well filled grain panicles and higher grain weight
- Resists insects because it allows rice to absorb soil nutrients naturally

Source: WASSAN-CSA-WWF Manual on SRI

Benefits of SRI

- Higher yields - Both grain and straw
- Reduced duration (by 10 days)
- Lesser chemical inputs
- Less water requirement
- Less chaffy grain %
- Grain weight increased without change in grain size
- Higher head rice recovery
- Withstand cyclonic gales
- Cold tolerance
- Soil health improves through biological activity

Disadvantages

- Higher labour costs in the initial years
- Difficulties in acquiring the necessary skills
- Not suitable when no irrigation source available

Conclusion

The System of rice intensification technique not only can save the water and cost but also will stabilize the environment as it involves the use of organic manures. From System of rice intensification highest growth parameters, grain yield and highest benefit cost ratio was recorded. As the SRI technique is best suitable for conserving the water and helps in cost cuttings for the usage of seeds for nursery and labour charges. SRI provides the farmer to grow the rice with less or minimal quantity of water. By adopting the SRI technique, the farmer can save the existing land from deterioration by reducing the use of chemical fertilizers and gets the highest yields.

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ADVANCED BACKCROSS QTL MAPPING

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Method of combining QTL analysis with variety development

- A strategy to identify and transfer beneficial QTLs from an unadapted line (e.g., land races, wild species) into an elite breeding line
- Effective in detecting additive, dominant, partially dominant, or overdominant QTLs.
- Exploits unadapted and exotic germplasm for the quantitative trait improvement without linkage drag accurately in a short period of time which was demonstrated in several crops.

Land races and wild germplasm: an under-utilized reservoir of genetic variation:

There is no shortage of genetic variation in nature. The wild ancestors and related land races of most crop plants can still be found in their natural habitats and national and international centers have been established to collect and maintain this material. For most major crop plants, germplasm banks contain tens of thousands of individual seed accessions. However, only a few of these accessions have actually contributed to the development of new elite varieties. Nevertheless, we maintain these collections with the tacit belief that the alleles contained in these wild and unadapted accessions will someday fuel crop plant improvement. The unfortunate reality is that, for the most part, we have been unable to exploit the majority of the genetic potential stored in germplasm repositories.

Problems with using unadapted germplasm to improve quantitative traits:

1. Linkage drag:

The productivity, uniformity and quality of modern, elite cultivars is based on decades of selection by breeders. As this elite germplasm becomes more differ- entiated from primitive cultivars, the option of utilizing wild or unadapted germplasm, which is likely to contri- bute many undesirable characters and require intensive selection to recover acceptable cultivars, becomes less and less attractive. For this reason, unadapted or wild species germplasm has been used mainly as a source of major genes for disease and insect resistance which can be incorporated via a backcross breeding scheme. Even in these cases, the transfer of the resistance genes has often been fraught with problems associated with link- age drag. Theoretical and empirical studies have shown that, even after 20 or more years of traditional breeding, a single gene transferred from a wild species will be associated with enough linked chromosomal DNA to contain more than 100 other, potentially undesirable genes (Young and Tanksley 1989; unpublished results).

Breeding to introduce polygenic characters from wild germplasm, where epistasis becomes a problem and linkage drag is compounded, has been generally avoided. Molecular linkage maps provide a method to reduce the problems with linkage drag by allowing selection for individuals containing recombinant chromosomes which break linkage drag. It has been estimated that the use of molecular maps can reduce linkage drag at least tenfold in a fraction of the time needed for traditional breeding (Tanksley *et al.* 1989). In tomato, the molecular linkage map has been used to identify varieties carrying minimal linkage drag around several disease resistance genes transferred from wild species (Young and Tanksley 1989; Messeguer 1991)

2. Having inferior phenotypes with respect to key quantitative traits:

While it is relatively straightforward to identify wild accessions that contain genes for resistance to pathogens, it is difficult to identify accessions that are likely to contain genes for the improvement of quantitative traits such as yield. In this respect, unadapted germplasm is almost always inferior to elite varieties. Report of occasional transgressive variation for yield in populations derived from crosses of elite lines to wild species suggests that, despite their inferior phenotypes, wild accessions do contain genes that can improve quantitative traits (Frey et al. 1981). Recent studies in tomatoes utilizing molecular markers have confirmed this hypothesis and have demonstrated that QTLs isolated from wild accessions, can substantially improve the phenotype of commercial tomato varieties for most quantitative. In these studies, molecular markers were used to screen the genome of the wild species *L. pennellii* for QTLs that modify both botanical and horticultural traits. Altogether more than 20 traits were studied. Regardless of the character, 10-50% of the QTLs detected in the wild species improved the trait of interest, even though the wild species phenotype was inferior to that of the cultivated parent. Specific QTLs for increased yield and soluble solids were subsequently transferred into the cultivated tomato using linked molecular markers.

Problems with balanced population:

- Undesirable QTL alleles from the unadapted parent occur in high frequency and can seriously reduce the ability to collect meaningful data on yield and other field performance traits.
- Epistatic interactions are statistically difficult to detect, yet are likely to occur in balanced populations
- For breeding purposes, it is desirable to identify QTLs not requiring epistatic interactions among donor alleles- a goal which is more difficult to achieve in balanced populations.
- Subtle (and often negative) pleiotropic effects may go unnoticed in balanced populations due to the large genetic and phenotypic variance created by the segregation of donor alleles in high frequency. A possible solution to the above problems would be to delay QTL analysis until an advanced generation (e.g., BC_2, BC_3 etc.).

Benefits of advanced backcross populations versus more balanced populations (e.g., F_2, BC_1, RILs) are:

- The genotype (and phenotype) of an average individual would be much more similar to the elite parent in an advanced backcross population than in a balanced population, making measurements of yield and other characters more meaningful.
- By waiting until an advanced generation, one could allow for phenotypic selection to further reduce the frequency of deleterious or undesirable alleles from the donor.
- Problems with deleterious characters (e.g., sterility, seed shattering, *etc*.), often encountered in balanced populations derived from wide crosses, can be reduced or eliminated.
- One would be less likely to detect QTLs with epistatic effects in advanced generations due to the overall lower frequency of donor alleles.
- QTL-NIL lines (Additional back cross generations) can be finally subjected to extensive field testing in replicated trials.
- Associated deleterious effects due to linkage drag are reduced in advanced generations since there have been more opportunities for useful meiotic recombination.

The general strategy of AB-QTL analysis is comprised of the following experimental phases:

- Generation of an elite unadapted donor hybrid,
- Backcross to the elite parent to produce BC_1 and BC_2 populations which are subjected to marker and/or phenotypic selection against undesirable donor alleles (e.g., for indeterminate growth habit, photoperiod responseor small fruit size),
- Molecular marker characterization of the BC_2 or BC_3 population,
- Generation of BC_3 or BC_4 families which are evaluated for agronomic performance and analysed for QTLs,
- Selection of target genomic regions containing useful donor alleles for the production of NILs in the elite genetic background using marker-assisted selection
- Evaluation of the agronomic performance of the NILs and elite parent controls in replicated environments
- QTL analysis using early generation hybrids has been successful in identifying favourable genes from wild species; however, much work remains in order to introduce and utilize the beneficial genes identified using these populations in a mainstream breeding program. Due to issues related to barriers to gene introgression commonly encountered with wild species such as segregation distortion, suppression of recombination, and linkage drag, the genetic improvement potential of many beneficial genes identified from wild species have not been realized. The AB-QTL approach, which advocates delaying QTL analysis until BC_2 or BC_3, was proposed as a strategy that in principle has the advantage of detecting and integrating favorable QTLs from wild species into elite breeding lines. In the last 15 years, AB-QTL populations have been created for most major crop species, and the examples presented herein have collectively demonstrated that this approach has made significant contributions in unlocking favorable alleles from the wild species parents.

In particular, the AB-QTL approach has shown that the genetic potential of exotic germplasm cannot be predicted based on phenotype alone. Because segregation has broken the donor genome into smaller components, the action of individual genetic loci is more clearly resolved than in earlier generation hybrids, resulting in more QTLs being identified and the genetic effects of individual loci more accurately estimated in the AB-QTL populations. Also, once a favorable QTL has been identified, NILs can quickly be developed with as little as one additional backcrossing to the recurrent parent when performed in conjunction with selection using linked DNA markers. These NILs can then directly be used as donor parents in cultivar development without much of the risks associated with standard interspecific gene introgression. Finally, with the target QTL becoming the major genetic source of variation due to the absence of other segregating QTLs, the NILs are a powerful resource for further genetic dissection of QTLs, including the eventual map-based cloning of the underlying gene, which has now been accomplished in a number of crop species.

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BRIEF ON CYTOKININ

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Cytokinins are **plant-specific chemical messengers (hormones) that play a central role in the regulation of the plant cell cycle and numerous developmental processes**. Cytokinins were discovered by F Skoog, C Miller, and co-workers during the 1950s as factors that promote cell division (cytokinesis) (Werner 2001)

Functions:

- Initiation of cell division
- Delay of senescence
- Use in tissue culture
- Counteracts apical bud dominance
- Induces flowering in SDPs
- Regulates cell division in shoot and roots

Cellular and molecular modes of cytokinin action:

The effects of cytokinin on plant growth and development is consistent with the involvement of signal transduction pathways with branches leading to specific responses.

Cytokinins are generally required for cell division of plant cells in vitro. Several lines of evidence suggest that cytokinins also play key roles in the regulation of cell division in vivo. Much of the cell division in an adult plant occurs in the meristems.

Genes and cytokinin relationship:

Localized expression of the ipt gene of Agrobacterium in somatic sectors of tobacco leaves causes the formation of ectopic (abnormally located) meristems, indicating that elevated levels of cytokinin are sufficient to initiate cell divisions in these leaves. Elevation of endogenous cytokinin levels in transgenic Arabidopsis results in over expression of the KNOTTED homeobox transcription factor homologs KNAT1 and STM-genes that are important in the regulation of meristem function.

Overexpression of KNAT1 also appears to elevate cytokinin levels in transgenic tobacco, suggesting an interdependent relationship between KNAT and the level of cytokinins.Over expression of several of the Arabidopsis cytokinin oxidase genes in tobacco results in a

reduction of endogenous cytokinin levels and a consequent strong retardation of shoot development due to a reduction in the rate of cell proliferation in the shoot apical meristem.

Elevation of endogenous cytokinin levels in transgenic Arabidopsis results in over expression of the KNOTTED homeobox transcription factor homologs KNAT1 and STM—genes that are important in the regulation of meristem function (Wang et al., 2006).

This finding strongly supports the notion that endogenous cytokinins regulate cell division in vivo. The same overexpression of cytokinin oxidase in tobacco led to an enhancement of root growth, primarily by increasing the size of the root apical meristem.Since the root is a major source of cytokinin, this result may indicate that cytokinins play opposite roles in regulating cell proliferation in root and shoot meristems

Meristem function:

Disruption of all the 3 cytokinin receptors in Arabidiopsis results in cytokinin insensitive plants that exhibit reduced cell division in both root and shoot apical meristem. The roots of these triple receptor mutants elongate for only a few days and then cease growth, a phenotype correlated with decreased cell division in the apical meristem (Chang et al., 2019).

In contrast, mutants that are only partially insensitive to exogenous cytokinin display an increase in root growth similar to that observed in plants overexpressing cytochrome oxidase.

Vasculature differentiation:

- Analyses of mutations in the cytokinin receptor mutations in the cytokinin receptor disrupt the development of the root vasculature.
- Known as cre1, these mutants have no phloem in their roots; the root vascular system is composed almost entirely of xylem.
- Further analysis revealed that this defect was due to an insufficient number of vasculature stem cells. That is, at the time of differentiation of the phloem and xylem, the pool of stem cells is abnormally small in cre1 mutants; all the cells become committed to a xylem fate, and no stem cells remain to specify phloem.
- Cytokinin plays a key role in regulating proliferation of the vasculature stem cells of the root.
- Cytokinin Regulate Specific Components of the Cell Cycle
- Cytokinins regulate cell division by affecting the controls that govern the passage of the cell through the cell division cycle.Zeatin levels were found to peak in synchronized culture tobacco cells at the end of S phase, mitosis, and G1 Phase.Inhibition of cytokinin biosynthesis blocks cell division , and application of exogenous cytokinin allows cell division to proceed.
- Cytokinins participate in regulation of the cell cycle and that they do so by controlling the activity of Cyclin-Dependent Kinases(cdk)

- cyclin-dependent protein kinases (CDKs), in concert with their regulatory subunits, the cyclins, are enzymes that regulate the eukaryotic cell cycle. The expression of the gene that encodes the major CDK, Cdc2 (cell division cycle 2), is regulated by auxin.
- Cytokinin has been linked to the activation of a Cdc25- like phosphatase, whose role is to remove an inhibitory phosphate group from the Cdc2 kinase This action of cytokinin provides one potential link between cytokinin and auxin in regulating the cell cycle: regulating the passage from G2 to M phase.

Regulation of CYCD3 gene by CK:

Cytokinins elevate the expression of the CYCD3 gene, which encodes a D-type cyclinIn animal cells, D-type cyclins play a key role in regulating the passage through the restriction point of the cell cycle in G1. D-type cyclins are key players in the regulation of cell proliferation (Dewitte et al., 2007)

In Arabidopsis, CYCD3 is expressed in proliferating tissues such as shoot meristems and young leaf primordia. In a crucial experiment, it was found that over expression of CYCD3 can bypass the cytokinin requirement for cell proliferation in culture

These and other results suggest that a major mechanism for cytokinin's ability to stimulate cell division is its increase of CYCD3 function.

Cytokinins Delay Leaf Senescence

- Leaves detached from the plant slowly, lose chlorophyll, RNA, lipids, and protein, even if they are kept moist and provided with minerals.
- This programmed aging process leading to death is termed senescence.
- Treating isolated leaves of many species with cytokinins will delay their senescence.

Role of cytokinin in regulating the onset of leaf senescence:

- Tobacco plants were transformed with a chimeric gene in which a senescence-specific promoter was used to drive the expression of the ipt gene.
- The transformed plants had wild-type levels of cytokinins and developed normally, up to the onset of leaf senescence.
- As the leaves aged, however, the senescence-specific promoter was activated, triggering the expression of the ipt gene within leaf cells just as senescence would have been initiated.

Cytokinins Promote Chloroplast Development:

Although seeds can germinate in the dark, the morphology of dark-grown seedlings is very different from that of light grown seedlings. Dark-grown seedlings are said to be etiolated.

Reason for etiolation: Instead of maturing as chloroplasts, the proplastids of dark-grown seedlings develop into etioplasts, which do not synthesize chlorophyll or most of the enzymes

and structural proteins required for the formation of the chloroplast thylakoid system and photosynthesis machinery.

Effect of cytokinin: If the etiolated leaves are treated with cytokinin before being illuminated, they form chloroplasts with more extensive grana, and chlorophyll and photosynthetic enzymes are synthesized at a greater rate upon illumination.

These results suggest that cytokinins along with other factors, such as light, nutrition, and development, regulate the synthesis of photosynthetic pigments and proteins. Cytokinins promote chloroplast development Cytokinins Promote Movement of Nutrients. Cytokinins influence the movement of nutrients into leaves from other parts of the plant, a phenomenon known as cytokinin-induced nutrient mobilization.

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