THE ROLE OF THE INOCULATION METHOD IN THE DEVELOPMENT OF AGRICULTURE

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ABSTRACT

Nowadays as we know population of Earth planet are about 8 billions people. As a result Global increases in food production achieved one of the urgent issues facing the scientists of the world is to meet this need, chemical methods are not always useful for human health, therefore, the production of products using useful microorganisms is one of the main directions of production in the 21st century. Inoculation processes dramatically reduce production costs: save space, reduce lead time.

Key words: *microbial inoculation, immunization, bacteria, pathogen, implanting, inoculating legumes, N-fixing bacteria, molecular farming.*

Global increases in food production achieved in recent decades have required large (15–20 times) increases in the useof synthetic pesticides to control pests, pathogens and weeds of crops (Oerke 2006) but the increasing use of synthetic pesticides is no longer sustainable. Strong consumer pressure has resulted in the with drawal of many synthetic pesticides, the lowering of maximum residue limits and changes in the regulatory environment that favour more environmentally benign control options. In addition, costs of development and registration of synthetic pesticides have been escalating, leading to significant reductions in development and launch of new chemistries (Glare et al. 2012) further limiting control options available to growers. The search for alternative solutions for agriculture has prompted researchers to take a second look at the range of microorganisms long known to provide benefits to agricultural production and is driving rapid growth in markets for biopesticides (Lehr 2010) and plant growth promoting microorganisms (Berg 2009). Regardless of the purpose for which beneficial microorganisms are applied to crops, they must be mass produced and applied in a way that optimises their functionality in the target environment. They have variously been delivered (mainly for research purposes) as liquids (sprays, drenches, root dips) or as dry formulations applied in furrow at time of planting. However, many of these approaches are not feasible on a large scale because of the amount of microbial inoculum needed, particularly in broad acre crops. Application of beneficial microorganisms to seeds is an efficient mechanism for placement of microbial inocula into soil where they will be well positioned to colonise seedling roots or make contact with soil dwelling invertebrate pests that feed on plant roots.[1]

Application of beneficial microorganisms to seeds is not a new idea; the inoculation of legumes with N_2-fixing bacteria has a long history and underpins the widespread use of legumes of global significance in terms of world food supply (Graham and Vance 2003). However, despite the long history of inoculation of legumes and clear laboratory demonstration of the ability of a wide range of other beneficial microorganisms to improve crop performance, there are still very few commercially available microbial seed inoculants.[1]

Inoculation is the act of implanting a pathogen or other microbe or virus into a person or other organism. It is a method of artificially inducing immunity against various infectious diseases. The term "inoculation" is also used more generally to refer to intentionally depositing microbes into any growth medium, as into a Petri dish used to culture the microbe, or into food ingredients for making cultured foods such as yoghurt and fermented beverages such as beer and wine. This article is primarily about the use of inoculation for producing immunity against infection. Inoculation has been used to eradicate smallpox and to markedly reduce other infectious diseases such as polio. Although the terms "inoculation", "vaccination", and "immunization" are often used interchangeably, there are important differences. Inoculation is the act of implanting a pathogen or microbe into a person or other recipient; vaccination is the act of implanting or giving someone a vaccine specifically; and immunization is the development of disease resistance that results from the immune system's response to a vaccine or natural infection.[2]

The earliest hints of the practice of inoculation for smallpox in China come during the 10th century.[4]

Until the early 1800s inoculation referred only to variolation (from the Latin word variola = smallpox), the predecessor to the smallpox vaccine.[5] The smallpox vaccine, introduced by Edward Jenner in 1796, was called cowpox inoculation or vaccine inoculation (from Latin vacca = cow). Smallpox inoculation continued to be called variolation, whereas cowpox inoculation was called vaccination (from Jenner's term variolae vaccinae = smallpox of the cow). Louis Pasteur proposed in 1861 to extend the terms vaccine and vaccination to include the new protective procedures being developed. Immunization refers to the use of vaccines as well as the use of antitoxin, which contains pre-formed antibodies such as to diphtheria or tetanus exotoxins.[2] In nontechnical usage inoculation is now more or less synonymous with protective injections and other methods of immunization.[8]

Inoculation also has a specific meaning for procedures done in vitro (in glass, i.e. not in a living body). These include the transfer of microorganisms into and from laboratory apparatus such as test tubes and petri dishes in research and diagnostic laboratories, and also in commercial applications such as brewing, baking, oenology (wine making), and the production of antibiotics. For example, blue cheese is made by inoculating it with *Penicillium roqueforti mold*, and often certain bacteria.[7]

Inoculation is the process of applying Rhizobium bacteria to legume seed to form a symbiotic relationship with the developing plant. Bacteria (*Rhizobium and Bradyrhizobium*) are capable of fixing atmospheric nitrogen (N) into forms usable by plants. The N-fixing bacteria are of two general kinds—symbiotic and non-symbiotic. The non-symbiotic group consists of free-living organisms, whereas the symbiotic group cannot function without the aid of a host plant. The association between the host plant and the symbiotic bacteria is mutually beneficial in that the plant furnishes the necessary energy, and the bacteria uses this energy to fix atmospheric N that can be used by the host plant. Since the discovery of BNF in the late 19th century, research works have been reported on its potentiality as an alternative to inorganic N-fertilizer in agriculture (Bala, 2011a; N'cho et al., 2013). This knowledge soon led to the practice of inoculation, with early adoption achieved by transferring soil from field to field, or soil to seed before planting. However, this was quickly replaced by the use of pure cultures on agar slants, and later as broths (Bala, 2011a). Hence, rhizobia inoculants - used to deliver nitrogen fixing bacteria (collectively termed rhizobia), have been on the commercial market for over 100 years in many developed countries (Nelson, 2004; Giller, 2008; GRDC, 2013). There are nearly 70 million tonne of soybean inoculated annually with *Bradyrhizobium japonicum* in the USA, in addition to 34 and 53 million tonne in Argentina and Brazil, respectively (Bala, 2011a). The benefits of inoculation coupled with the application of deficient nutrients, mostly phosphorus vary with location and soils .Inoculating legumes with rhizobia has been used to achieve substantial increases in legume nodulation, grain and biomass yield, nitrogen fixation and post-crop soil nitrate levels over this period. These gains are usually highest when the inoculated legumes are grown in nil-rhizobia or low-rhizobia soils, but marginal in soils already containing high number of compatible rhizobia (GRDC, 2013)

With a few exceptions, the plants with which the symbiotic N-fixing bacteria are associated belong to the legume family. The development of nodules on the roots of a legume is evidence that the symbiotic bacteria are present. Since the symbiotic organisms are usually associated with legumes and result in the formation of nodules, the organisms are often called "legume bacteria" or "nodule bacteria." The bacteria genus "Rhizobium" is the common "legume bacteria."

Methods for "inoculating" seeds include:

1) Applying a commercially prepared culture of the proper strain of bacteria to the legume seed or in the seed furrow at planting, or 2) spreading soil from a field in which the legume recently has become inoculated and has grown successfully. However, the disadvantages of using soil outweigh the advantages to such an extent that commercial cultures are generally used either on the seed or sprayed in furrow or a similar manner. When using commercial inoculants, the following rules for successful inoculation should be observed:

1. Purchase fresh inoculant from the proper cross-inoculation group. Before completing purchase, note the expiration date printed on the container and make sure that the bacteria culture is of a strain that will inoculate the legume to be planted. See Table 1 for legumes included in each cross-inoculation group. Store culture in a cool, dry place until it is to be used.

2. Inoculate if there is any doubt as to whether bacteria of the proper strain are present in the soil. If peanuts or soybeans have a long history of being planted in the field, less response to inoculants may occur.

3. For powder inoculants, follow directions for each crop. Put a sticking compound on the seed, then add inoculant and mix well with seed. If seed becomes too wet, allow to dry before putting into a seed hopper for planting. Do not allow direct sunlight to hit the inoculated seed. During planting apply granular or liquid inoculants in the seed furrow at the manufacturers' recommended rates.

4. Do not allow seed to contact caustic lime or soluble fertilizers. Some seed-protecting chemicals are incompatible with legume bacteria. Molybdenum may aid in nodule formation on soybeans planted on mineral soils that have not been limed to the optimum pH. Do not purchase inoculant pre-mixed with molybdenum because the bacteria may die in such pre-mixes. If needed, these materials should not be mixed with the inoculant until just prior to planting.

5. Plant inoculated seeds at once (within four hours) into moist soils and cover them immediately. Most planters have press wheels that pack the soil for good soil-seed contact which is important for fast germination and survival of the bacteria.

6. Plant when both soil temperature and moisture are favorable for quick germination of the legume and favorable for the survival of the bacteria. .[5]

Agriculture is facing new challenges, with global warming modifying the survival chances for crops, and new pests on the horizon. To keep up with these challenges, gene delivery provides tools to increase crop yields. On the other hand, gene delivery also opens the door for molecular farming of pharmaceuticals in plants. However, towards increased food production and scalable molecular farming, there remain technical difficulties and regulatory hurdles to overcome.

Plants are one of the two main sources of food (animals being the other) and with increasing demand for food, pollution in the environment, chemical threats, as well as natural disasters such as droughts and fires, plant engineering is essential for food production. According to a meta-analysis done in 2021, it is estimated that the demand for food will rise by 35–46 percent between 2010 and 2050 (van Dijk et al., 2021). Plant-based expression systems are also an emerging platform for production of life-saving pharmaceuticals (Chung et al., 2021). This concept of molecular farming was introduced in 1986 for the production of human growth hormone (hGH) in transgenic tobacco and sunflowers (Barta et al., 1986), and achieved years later for an IgG1 antibody in transgenic tobacco (Hiatt et al., 1989). More recently, the antibody cocktail ZMapp, which was used to treat Ebola patients during the 2014 epidemic, was produced in Nicotiana benthamiana by Kentucky BioProcessing, Inc. (Zhang et al., 2014), while Medicago Inc. uses the same plants to produce seasonal influenza vaccines (Ward et al., 2020). Both companies have also produced COVID-19 vaccine candidates: while Medicago produced a virus-like particle (Ward et al., 2021), Kentucky BioProcessing produced a platform vaccine by conjugating the RBD domain to tobacco mosaic virus (TMV; Royal et al., 2021). Molecular farming has grown out of its infancy and demonstrated its relevance for biopharmaceutical manufacturing.[6]

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