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Dipartimento di Agronomia, Animali, Alimenti,
Risorse Naturali e Ambiente

Master Degree in Sustainable Agriculture

EFFECTS OF A NEW BIOSTIMULANT ON VEGETATIONAL INDEXES, YIELD AND QUALITY IN COMMON WHEAT

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Abstract:

Wheat is the most cultivated crop in the world with a high nitrogen requirement. In order to reduce nitrogen leaching and abiotic stress risks related to climate change, new agronomic practices are currently being developed to maximise nitrogen use efficiency and crop yield and quality. Among these, the use of bio-stimulants is spreading worldwide in many crops and may replace late season foliar nitrogen supply in wheat.

In this thesis, a new bio-stimulant obtained from sage and husk rice extracts was applied as foliar spraying in three common wheat varieties (i.e., var. Bologna, Vivendo and Solehio) at flowering stage and its effects were compared with untreated controls and two benchmark commercial bio-stimulants obtained from protein hydrolysates.

The trial was arranged at the Experimental farm of the University of Padova at Legnaro during the 2020-21 season, following a completely randomised block design with 3 replicates. After application of bio-stimulants, the canopy greenness was monitored during the grain filling by weekly detection of NDVI and SPAD, while at harvest grain yield was quantified through a plot combine harvester. Quality and bread-making parameters were measured by NIRS and the Chopin alveograph, respectively.

Similarly, to the two benchmarks, the new bio-stimulant significantly delayed leaf senescence of wheat, that is commonly observed with the approach of ripening. Compared to the untreated controls, higher NDVI values were found with all the three bio-stimulants for var. Bologna, and the maintenance of high values of leaf chlorophyll content (SPAD values) for longer time in var. Vivendo and Solehio, especially with the new bio-stimulant. The maintenance of canopy greenness for a longer period than the control led to significant increases in protein content, wet gluten and the Zeleny index, with more marked improvements in var. Bologna compared to Solehio and Vivendo. The results on yield were contrasting, with a slight decrease in Bologna and Vivendo (more protein-rich varieties) and a slight increase in Solehio, regardless of the type of bio-stimulant applied. It is believed that yield response was greatly affected by the particular climatic conditions in 2021, with abundant rains and low temperatures in April and May, suggesting the need to repeat the trial for a second year.

The qualitative improvements obtained by the bio-stimulants linked to proteins and gluten did not translate into evident variations in the rheological quality of the doughs. From the nutraceutical point of view, the possible increase of the content of antioxidants (phenolic acids) in flour of some varieties with one of the two benchmarks is very interesting.

Introduction:

Wheat is a versatile crop that can be grown in a variety of climates worldwide. It can be grown in a wide range of climates, from the humid and warm to the dry and cold. Wide-ranging adaptation has undoubtedly been made possible by the genome's complexity, which gives the crop a great deal of plasticity. C3 plants, like wheat, thrive in cool climates because of their ability to convert carbon dioxide into glucose. Physiology, growth and development of this crop have been studied extensively in the past and are now fairly well understood (Wheat growth and physiology - Acevedo et al., 2006).

There has been a lot of research to identify functional amendments to promote plant growth, productivity, and quality and to help plants resist to environmental stresses. Today's horticultural industry must balance high productivity with worldwide needs for environmentally responsible crop management approaches. Chemical fertilizers and pesticides are limited in organic agriculture (Pascual et al., 2018), hence various plant amendments are needed. In degraded agricultural areas and climatic uncertainty, bio stimulants can also be an option. Vernieri et al (2006), see hydroponics and bio-stimulants as an environmentally friendly way to raise greenhouse crops. Use of natural plant bio-stimulants (PBs) to improve flowering, plant growth, fruit set, crop productivity, and nutrient use efficiency (NUE), as well as to increase tolerance against a wide range of abiotic stressors, is a promising and environmentally friendly innovation that could replace harmful pesticides and fertilizers even in field crops (Colla and Rouphael, 2015).

1.1.WINTER WHEAT [Triticum aestivum]:

Bread wheat, or *Triticum aestivum*, is a species in the genus *Triticum* and the tribe *Triticeae* of the family *Poaceae* (e.g., *Gramineae*). A total of 18 different genera make up the tribe *Triticeae;* they are further subdivided into the *Triticinae* and the *Hordeinae*. *Triticum, Aegilops, Secale, Agropyron*, and *Haynaldia* are the most important genera in the *Triticinae* subgroup (Körber-Grohne, 1988).

Winter wheat is one of several types of wheat that, when planted in the fall, develop into young plants that remain vegetative throughout the winter and resume growth in the spring (winter type). Traditional classifications for wheat include also "spring" types, referring to the time of sowing, that do not require vernalization. Winter wheat requires anywhere from 30-60 days for vernalization, the process by which the plant adjusts to the low temperatures of winter (0° to 5 °C; 32–41 °F). In this physiological setting, the ear begins to develop(Curtis et al., 2002).

Winter wheat, grown in the Northern Hemisphere, is sown from September to November and harvested in the summer or early fall. In some areas, the winter-wheat crop might "complete" in a year and be ready for harvest (like Chile). Winter wheat crops often yield higher than spring wheat crops because of the longer cycle (Oulton & Randall, 2021).

In the Southern Hemisphere, planting and harvesting of spring wheat (which does not require a period of vernalization) can take place in the fall (November–December) and early spring (April–May) of the following year in regions with mild winters, such as South Asia (India, Pakistan, Nepal, Bangladesh), North Africa, the Middle East, and the lower latitudes (e.g., Sonora, Mexico). Although it is sown in the fall and matures in the winter, this spring wheat is often incorrectly classified as "winter wheat." (Oulton & Randall, 2021).

Higher levels of gluten protein can be found in hard winter wheats compared to other types of wheat. They are ground into flour for yeast breads or combined with soft spring wheats to create all-purpose flour, an ingredient in countless baked goods. Specialty or cake flour is milled exclusively from soft wheat (Winter wheat - Wikipedia, 2016).

1.1.1 World wheat production:

Wheat is the second most widely grown cereal crop in terms of both grain area. In the 2020/21 marketing year, global wheat production reached an estimated 772 million metric tons. As compared to the previous year, this represented an increase of around 10 million tons. Global wheat inventories are forecast to rise, ultimately reaching 294 million metric tons by 2021 (Shahbandeh, 2022) (Figs. 1-5).

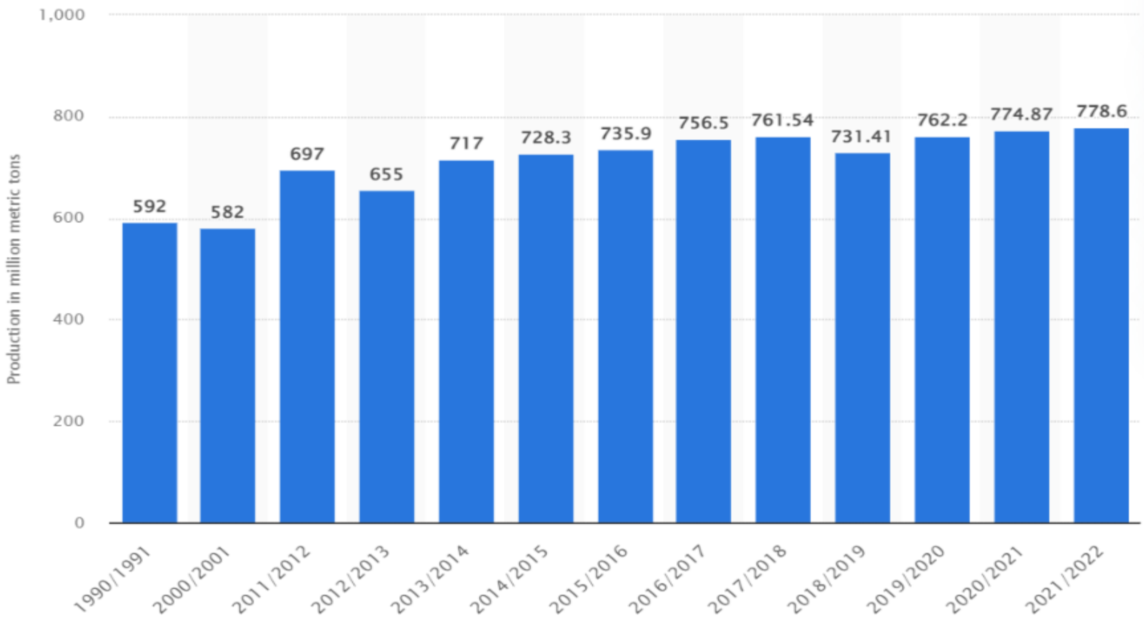


Fig1: Global wheat production from 2011/2012 to 2021/2022 (in metric tons)

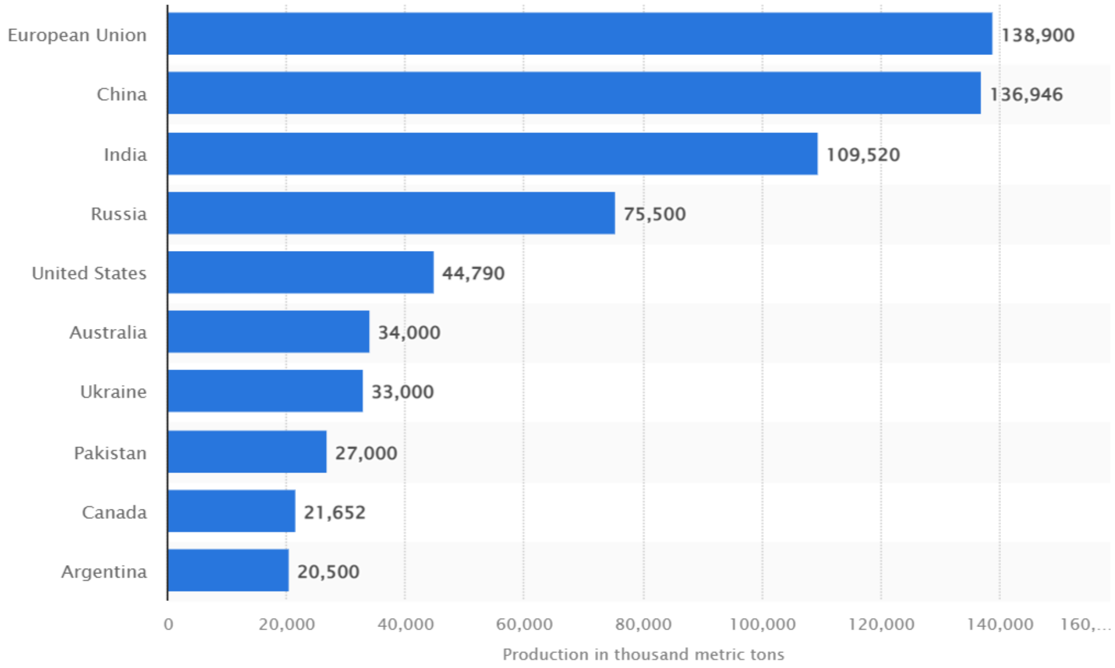


Fig 2: Top 10 wheat producing Countries in 2021/22

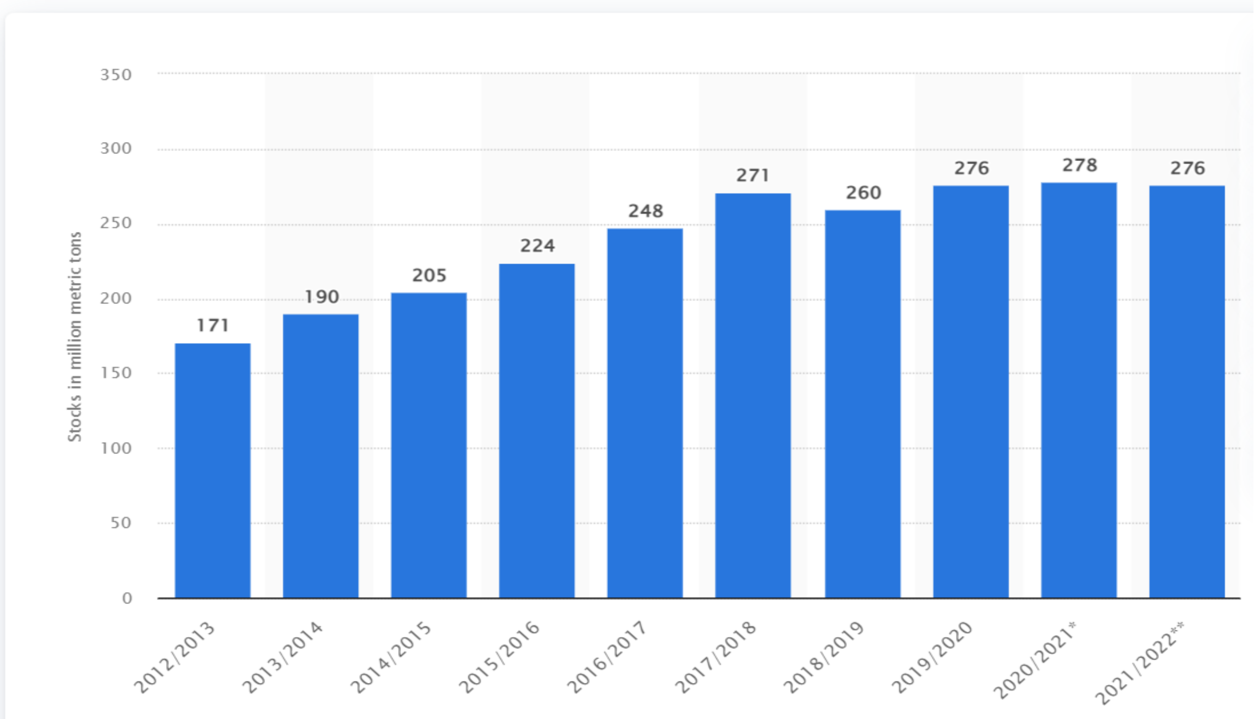


Fig 3: 2012/13 to 2021/22 wheat global stocks

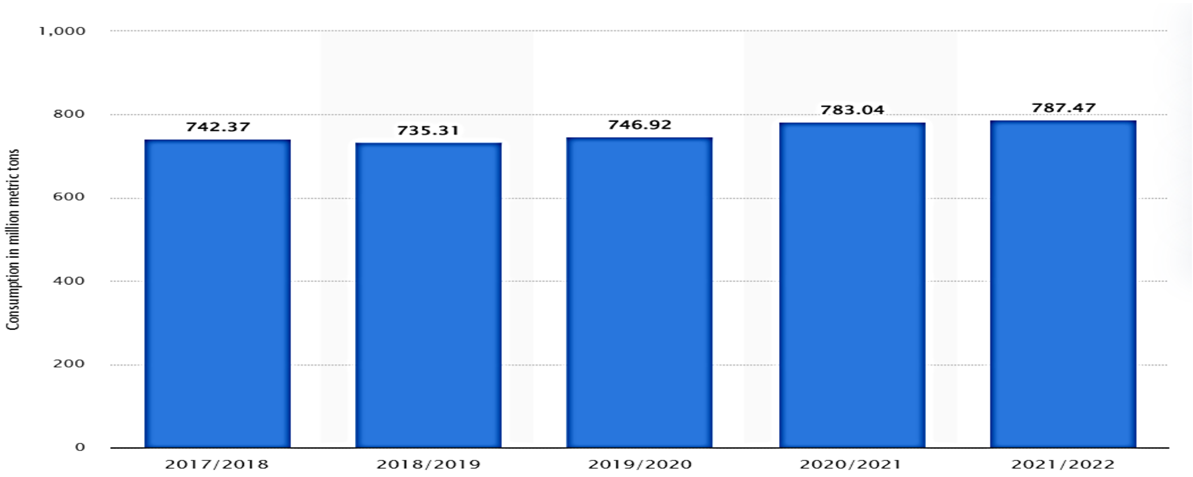


Fig 4: Worldwide wheat consumption from 2017/18 to 2021/22

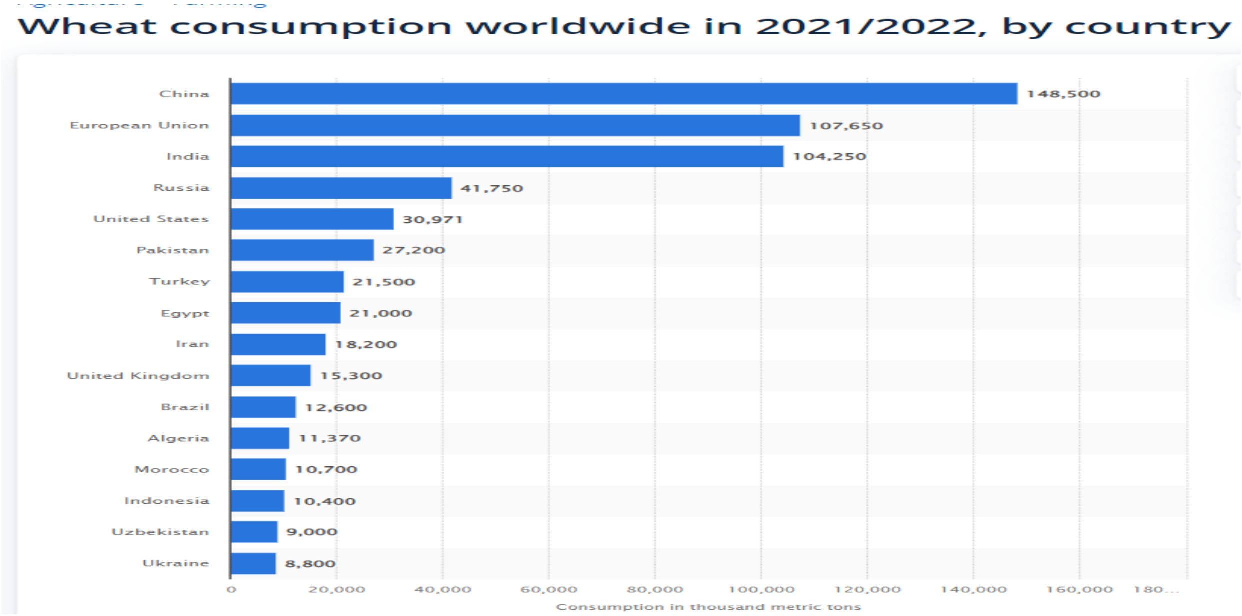


Fig 5: Worldwide wheat consumption by Country in 2021/22

1.1.2 Origin and botanic classification of winter wheat:

Modern wheat may be traced back over 10,000 years; it is a staple crop for millions of people. Historically, the Fertile Crescent in southwest Asia was the site where wheat was first farmed by humans. Ancient wheat cultivation was first documented in the Levant and Turkey. An estimated 10,000 years ago, in the fertile crescent, wild einkorn and emmer wheat were tamed and domesticated. Mutant forms with tough ears that remained linked to the ear during the harvest process and larger grains were selected by farming wild grasses and continuously harvesting and resowing the grains. Selection for these traits was crucial to the domestication of crops (cs.mcgill.ca, 2007).

It was during the Neolithic era that wheat farming began to extend outside of the Fertile Crescent. Ethiopia, India, Ireland, Spain and the Middle East had access to wheat by 5,000 B.P. and by the 1,100s, it had made its way to China (cs.mcgill.ca, n.d.). Carbonized grains reaching back to at least 6,750 B.C. have been discovered in Iraq, with several more discoveries in Eastern Mediterranean countries being just slightly later. Wheat apparently swept throughout Europe no later than the Stone Age (Magness et al., 1971).

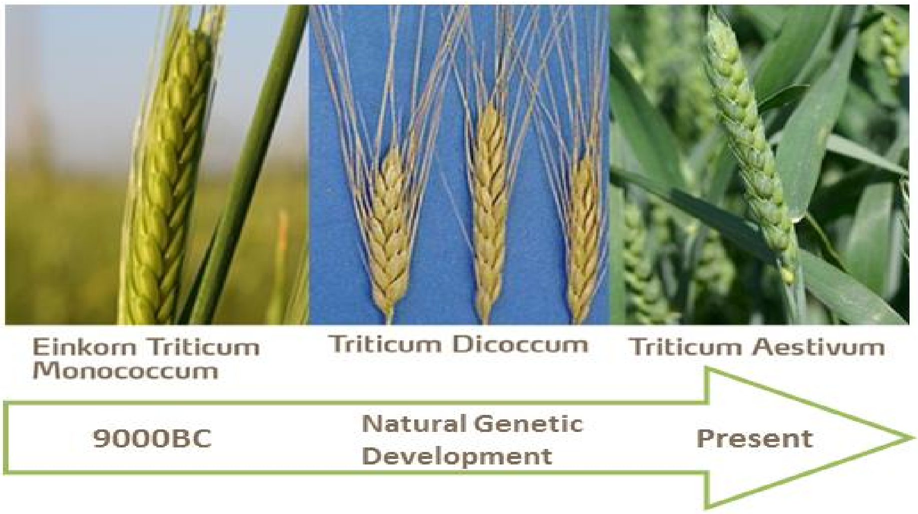


Fig 6: Evolution of wheat (yara.co.uk, 2017)

Emmer was the result of a natural hybridization between *Triticum urartu* (which is closely related to wild einkorn (*Triticum boeoticum)* and an *Aegilops* species, therefore this was an additional genetic development in the evolution of grasses. They were both diploids, thus the resulting wheat was a tetraploid, or a plant with four sets of chromosomes. Like *Emmer* wheat, durum is a tetraploid that evolved via natural hybridization. As time went on, new varieties of wheat came to predominate as farmers continued to pick out the ones with desirable characteristics from their fields.

Spelt and Common bread wheat became the go-to options over time. Emmer wheat and the wild goat-grass *Aegilops tauschii* naturally hybridised to create these new varieties. Hybridization has resulted in a hexaploid form of the tetraploid, with six sets of chromosomes (i.e., 42 chromosomes) rather than the 14 chromosomes seen in the original species. Biotechnology has been exploring how genetic management can be sped up and made more efficient through very precise gene editing since, while incredibly effective, this 'natural' genetic evolution has taken a very long period (yara.co.uk, 2017). Still, it is possible that ancient wheat looked somewhat different from modern wheat, with its kernels being significantly smaller. The first humans to domesticate wheat likely searched for plants with unusually large kernels, as they would yield a greater harvest from a given stalk (Charles, 2016).

Classification:

Wheat varieties can be sorted in two ways: either by type, based on the genetics of the variety, or by classification, depending on the characteristics of the variety.

- Colour (e.g., red, yellow, white)
- Planting Season: wheat is sown in the spring and harvested in the early fall; winter wheat is planted in the fall and harvested in the summer.
- The grain can be classified into three distinct types based on its characteristics: durum, hard bread wheat, and soft wheat.

Major cultivated species of wheat:

- Common wheat or Bread wheat (*T. aestivum*): an hexaploid species that is the most widely cultivated in the world.
- Durum (*T. durum*): the only tetraploid form of wheat widely used today, and the second most widely cultivated wheat.
- Einkorn (*T. monococcum*): a diploid species with wild and cultivated variants. Domesticated at the same time as emmer wheat, but never reached the same importance.
- Emmer (*T. dicoccum*): a tetraploid species, cultivated in ancient times but no longer in widespread use.
- Spelt (*T. spelta*): another hexaploid species cultivated in limited quantities in north Europe.

Fig 7: Cultivated wheat species (eatwheat.org.)

1.1.3 Morphology:

Stem: There is a main stem in a wheat plant, from which the leaves radiate outward. It contains a node, a hollow internode, a leaf, and a tiller bud in the leaf axil (Kirby, 2002). Leaf sheath wraps around stem, supporting shoot (Setter & Carlton, 2000). Wheat stems end in ears.

Leaf: The sheath and blade originate from independent meristems. At the leaf's base, where it meets the sheath, are the ligule and auricles (Kirby, 2002). All even-numbered leaves are on one side of the plant, and odd-numbered leaves are on the opposite side (Setter & Carlton, 2000). Spring wheat variants have longer leaves from the base to the flag leaf (Kirby, 2002).

Three leaf tissue types exist. On the underside of the leaf, fewer cell types make up the epidermis. The epidermal layers are waxed. The mesopyll is surrounded by epidermal layers and vascular tissue (Kirby, 2002).

Tillers: A wheat plant's tillers are the side shoots that grow out from the main stem (Kirby, 2002). Leaves grow on both the top and bottom of the main stem, and an ear can form at the very top of the plant (Setter & Carlton, 2000). Not all tillers will develop into mature plants capable of producing an ear, and this is thought to be due to struggles to find sufficient light and nutrients (Kirby, 2002).

The Ear: Two rows of spikelet's form a wheat ear and the floret-containing spikelet's are placed on opposing sides of a central rachis (Setter & Carlton, 2000), like the leaves on the central stem. Lemma and palea contain the carpel (ovary and stigmas) and three stamen and anthers (Setter & Carlton, 2000).

Each anther has four pollen-containing loculi (Kirby 2002). Each anther lobe includes microspore mother cells. Tapetum surrounds each column (Lersten, 1987). After each division of microspore mother cells, a cell plate forms. The tetrad is forced against the tapetum. Tapetal nuclei divide mitotically during meiosis, creating binucleate cells. The callose that isolates each meiocyte dissolves during meiosis, but the microspores remain on the tapetum. This continues until each pollen grain has a single pore at the tapetal contact point. Grass tapetum and pollen continually interact.

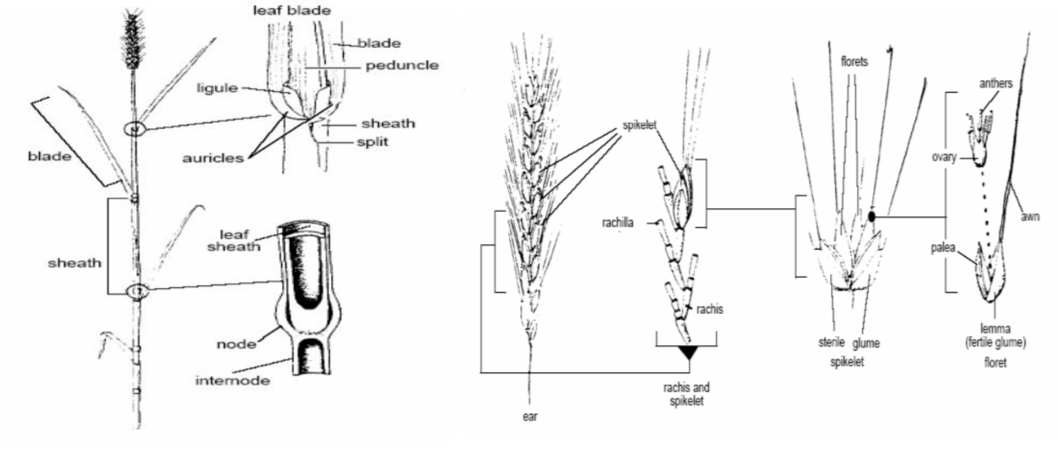


Fig. 7. Morphology of the wheat ear showing the structure of the spikelets and florets

1.1.4 Vegetative and reproductive phases:

Growth Stages from Germination to Maturation :

Winter wheat growth is staged. Germination leads to seedlings, then tillering, overwintering, jointing, booting, heading, and flowering. Milk, soft dough, hard dough, and physiological maturity are stages of grain maturation. Last, the grain loses moisture until it is ready to harvest.

A. Germination and Seedling Emergence:

High-quality seed is key to a strong stand. A minimum germination rate of 85% and test weight of 56 lb per bushel (i.e., about 0.72 kg L^{-1}) are required. Because of identical volume and weight, the advantages of planting a large seed over a typical, plump seed are lessened.

Wheat germinates by absorbing water and oxygen and soil must be wet and warm. When a seed germinates, an embryo grows into the plant's root and protective covering (scutellum) (its first leaf). Endosperm carbohydrates and proteins are also needed for germination and growth.

The coleoptile helps a seedling break ground 5 to 7 days after planting. When seed is placed deeper than the coleoptile's elongation distance allows, there are no seedlings and poor stand. Due to short coleoptiles and semidwarf wheat plants requires shallower planting.

Seed germination is related to soil depth. Deep seeding may be necessary because of dry soil. If soil moisture runs out before seedlings sprout, as could happen after mild showers, seedling survival depends more on the stage of germination than the dry interval. Two-day-old seedlings can tolerate being dried out and continue developing until rehydrated.

A radicle is one of a wheat seedling's primary roots and plant dies, if wheat's primary roots does not produce secondary roots. Wheat plants only have primary roots in the main stem, which can last the plant's entire life. Crown roots, also called secondary roots, are formed by a wheat seedling after it emerges from the soil and make up the plant's root system for the rest of its life. All tiller roots and many stem roots are secondary. A plant's roots anchor it and collect nutrients and water from the soil. Some roots may go 7 feet (2.1 m) below the surface, although most are in the upper 6 inches (15 cm) (the plough layer). Water and minerals are absorbed by root hairs. Wheat's drought resistance comes from its deep, widely spread roots and long root hairs.

B. The Growing Point and Seedling Growth:

Wheat is unique among plants in that all of its above-ground development originates from a single meristem, the growing point. The developing bud is a miniature wheat stem with nodes, internodes, and the wheat ear. Until spring, when the plant "differentiates" and begins to grow and head, it is safe from predators underground.

The third leaf on a seedling is the first foliage leaf and it is the site of photosynthesis, along with the other leaves of the plant's foliage, which can number anywhere from five to seven in spring wheat and eleven to fifteen in winter wheat. Wheat's chloroplasts are small structures within the cells that use sunlight, water, and carbon dioxide to make food and energy. The wheat plant relies exclusively on photosynthesis to produce its foliage and grain. Wheat's nodal buds create tillers, which are secondary branches. Sowing pace, soil moisture and fertility, temperature, and variety affect the number of tillers. Three to six tillers are usual for a winter wheat plant under normal conditions. Soon after the seedling emerges from the soil, tillers form and follow the same developmental route as the main stem.

Planting early, giving nitrogen fertiliser, and watering if necessary (in dry soil) all increase tillering. Planting later reduces tillering and demands larger seeding rates to compensate. Only in the fall do tillers form, and they don't grow until the spring after winter.

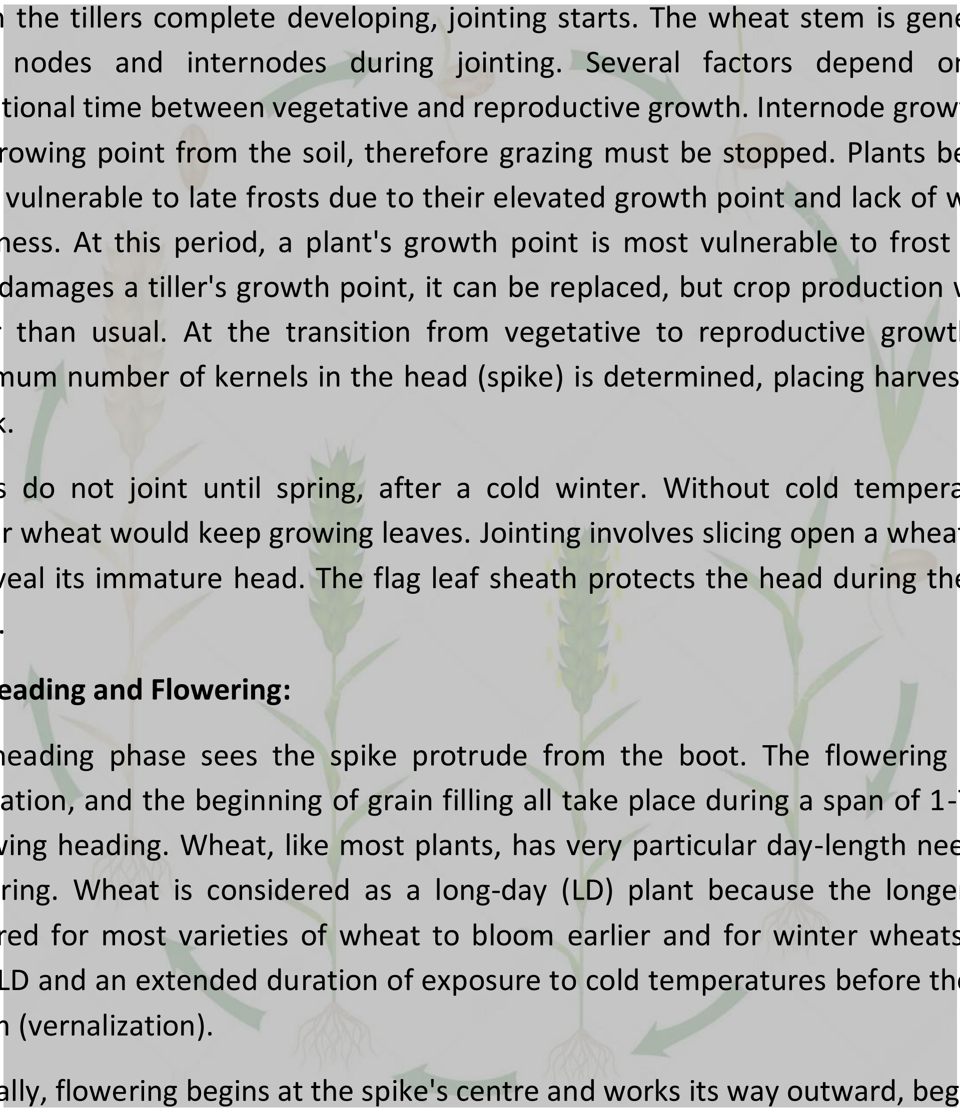
Overwintering:

Wheat plants become extremely cold hardy as a result of shorter days and dropping temperatures in the autumn. There is also a transition from an erect to a prostrate kind of development that occurs in most species. Hardiness tends to peak in the beginning of winter and progressively decline during the season. Late winter is when cold fronts do the most damage, after warm weather have caused plants to lose their hardiness. Snow is a great shield because it insulates plants from bitter cold and prevents them from waking up during brief warm periods. The growth point, the most vital component of the plant, spends the winter approximately an inch (i.e., 2.5 cm) beneath the soil's surface, where it receives some protection. When the growing point is damaged, the entire plant dies. When the growing point is frozen, it goes from looking white and turgid to looking brown and wilted. Mild winters allow leaves to remain green, although freezing or "burning" of leaves by cold has minimal effect on production.

C. Tillering:

Wheat "greens up" and resumes growth when the weather warms up in late winter. As the sheaths, or coverings of the leaves, get longer, the tillers that were started the previous fall grow quickly and revert to an upright form. Top-dressing with nitrogen fertiliser at this time will encourage the growth of the tillers, which will yield the majority of the grain come harvest time. At this point, the growing points are still safely tucked away underground, but the tillers may still be stunted by drought and other pressures. Using an herbicide like 2,4-D at this time is not recommended since it stunts the growth of the tillers.

D. Jointing and Booting:



When the tillers complete developing, jointing starts. The wheat stem is generated from nodes and internodes during jointing. Several factors depend on this transitional time between vegetative and reproductive growth. Internode growth lifts the growing point from the soil, therefore grazing must be stopped. Plants become more vulnerable to late frosts due to their elevated growth point and lack of winter-hardiness. At this period, a plant's growth point is most vulnerable to frost and if frost damages a tiller's growth point, it can be replaced, but crop production will be lower than usual. At the transition from vegetative to reproductive growth, the maximum number of kernels in the head (spike) is determined, placing harvest yield at risk.

Plants do not joint until spring, after a cold winter. Without cold temperatures, winter wheat would keep growing leaves. Jointing involves slicing open a wheat stem to reveal its immature head. The flag leaf sheath protects the head during the boot stage.

E. Heading and Flowering:

The heading phase sees the spike protrude from the boot. The flowering stage, pollination, and the beginning of grain filling all take place during a span of 1-7 days following heading. Wheat, like most plants, has very particular day-length needs for flowering. Wheat is considered as a long-day (LD) plant because the longer days required for most varieties of wheat to bloom earlier and for winter wheats need both LD and an extended duration of exposure to cold temperatures before they can bloom (vernalization).

Typically, flowering begins at the spike's centre and works its way outward, beginning 1 or 2 days before it does on the tillers. When the yellow anthers appear outside the florets, flowering is complete. The final count of spike-forming kernels is settled upon at this point. Wheat is extremely self-fertile and resistant to most stressors except frost during flowering. When temperatures drop just a little below freezing, the male floral parts die off quickly, and the florets are rendered sterile. However, due to differences in flowering, just a portion of the spike may be sterilised, leading to grain formation either at the spike's core or its tips.

F. Maturation and Ripening:

After flowering, grain grows immediately and reaches its maximum size (not weight) in 2 weeks. Endosperm is used to measure grain development. As the kernel grows, the endosperm thickens from soft to hard dough. The mature kernel has hardened, changed colour, and gained dry matter. The kernel is 30-35% water at its physiological apex.

Wheat kernel protein and starch are important. Photosynthesis sugars boost protein and starch during grain loading. Early in grain growth, proteins metabolize nitrogen. Even if yields are low due to improper kernel filling, the grain is protein rich. Drought and hot temperatures induce this. Grain protein is diluted if the grain fills, yields, and has a high-test weight. In appropriate growing circumstances, nitrogen fertiliser can raise grain protein levels.

Flag leaf, glumes, and awns remain active during grain-filling, while most other leaves die shortly after flowering. Photosynthesis in the awns, or beard, produces 10–20% of the grain's weight. In cooler, wetter climates, wheat leaves are more productive and less reliant on awns.

Maturation determines final kernel weight. Mild weather and vigorously growing leaves contribute to large, plump kernels and a good yield. High temperatures and foliar diseases like leaf rust (*Puccinia triticina*) cause kernels to shrink and harvests to fail. After physiological maturity, grain ripens. The biggest difference is the moisture content, which drops from 30–35% to 12–13%. To save the crop, mature grain must be collected quickly. Hail, lodging, and preharvest sprouting threaten mature grain.

(Wheat Production Handbook, 1997)

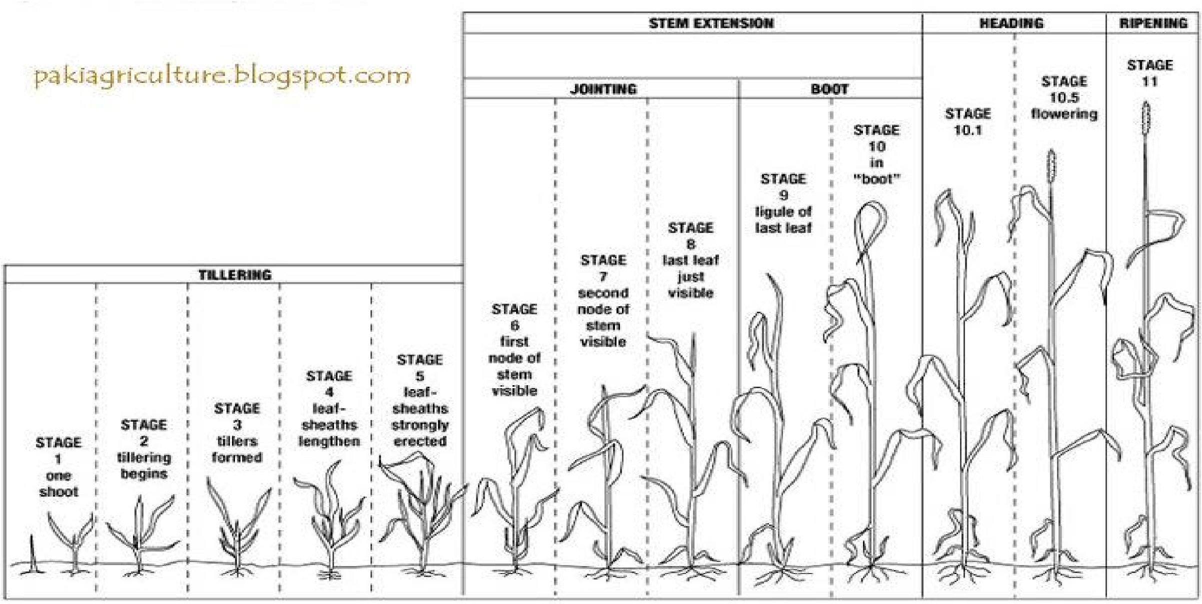


Fig 10: Growth stages of wheat plant (pakagrifarming, 2015)

1.1.5 Ecology and climatic requirements

A). Climatic conditions:

- Wheat is a crop that can thrive in a broad range of climates, although optimal are those temperate.
- For optimal development and harvest, a cool, dry, and clear climate is required.
- A range of 18 to 25 °C is ideal for development.
- Need between 750 and 1,600 millimeters of rain annually.
- Diseases like rust and root rot develop in hot and humid environments, therefore they're not good for the plants.
- Tillering is promoted by a combination of low temperatures and dew formation in the early growth stage.
- It's bad for the grain to fill in when there's frost, clouds, and cold temperatures.

B). Soil Requirements for Wheat Cultivation:

- Wheat may be cultivated in a wide variety of soils, from sandy deserts to dense clay.
- Irrigated wheat thrives in well-drained, fertile clay loam soils with a modest water holding capacity.
- The best conditions for growing wheat are deep, loose soil that can store monsoon rains for an extended time.
- Wheat cultivation is best suited to the black soils (agriinfo.in, 2018)

1.1.6 Cultivation management:

A. Important Characteristics to Consider Wheat varieties:

Maturity:

The early maturing types have a better chance of surviving the high winds, dryness, and rust. However, they are more vulnerable to late-spring freezes. Growers who tend to a large number of acres might better manage their yield by planting a mix of crop types at varying stages of maturity. Heads of varieties or hybrids that bloom before var. Jagger or after Var. Arapahoe may be damaged by frost (Kansas, USA), and late-season hot winds may reduce yields .

Winter Hardiness:

Wheat is most vulnerable to cold temperatures in the fall, before it has had a chance to harden, and in the spring, after growth has begun. There is a difference in how susceptible different varieties are to being damaged by freezing temperatures. There is a 1–9 scale used to rank winter hardiness, with 1 being the most resistant (best). This feature benefits greatly from a complementary set of varieties. Selecting a second variety with an emphasis on higher levels of winter hardiness is recommended if you want to plant a variety with a middling or bad rating for winter hardiness.

Disease and Insect Resistance:
When it is possible to do so, using genetic resistance to ward off pests and illnesses is an effective strategy. Wheat is vulnerable to a wide variety of pests and diseases, including hessian fly, wheat streak mosaic, soilborne mosaic, stem and leaf rusts, speckled leaf blotch, and tan spot. Losses can be reduced with the use of resistant or tolerant plant cultivars and good farming techniques.

Lodging and Shattering:
Wheat is often harvested using a combine, and it is best if harvesting occurs when the crop is fully mature; as a result, varieties that are resistant to lodging and shattering are highly sought after. Often, "semidwarf" wheats perform better in the field than "normal height" varieties. Some of the semi-dwarfs may have trouble producing adequate stands in arid areas where deep planting is required due to their short coleoptiles (sprouts).

Grain Quality:
Bread is the most common product made from wheat. Because of this, it is crucial that the grain meet the standards required by the millers and bakers. Variety and climate play equally important roles in determining final product quality. Since quality matters, growers need to keep it in mind while choosing cultivars.

Acid Tolerance:
The over-application of nitrogen fertilisers is turning many soils more acidic. Soils with a low pH have more free aluminium, which can damage the root tips and reduce plant vitality. As a wheat farmer, should know how acidic or basic the soil is. Different types of wheat have varying degrees of tolerance for acidic soils.

Coleoptile Length:
The short coleoptiles of many modern semidwarf types prevent them from sprouting from deeply planted containers. Standardized coleoptile length ratings are based on the Learned variety, which is considered a gold standard in the breeding industry. Farmers need to be careful about how deep they put their seeds of kinds with shorter than 3-inch (7,6 cm) coleoptiles. (Wheat Production Handbook, 1997)

B. Planting Practices:

Seed Quality:

Using high-quality wheat seed ensures successful planting, germination, and stand development. High-quality seed has a high germination rate, true-to-variety composition, and no crop seeds, weeds, foreign material, or disease. In unfavourable planting conditions, such as dry soils, deep planting, or late planting, seed quality might affect forage and grain yield. Farmers can affect forage and grain harvests by planting high-quality seed.

Many people rely on the grain's test weight to judge its quality; however, this is not necessarily correct because little seed that packs well can have a high test weight. Producers who rely on test weight should sow only seed weighing more than 57 pounds per bushel (about 0.73 kg L^{-1}). High TKW is a stronger indicator of seed quality. A seed lot should have at least 30 g TKW, or 15,200 seeds per lb. These cultivars require the largest seed possible and a germination rate of at least 85%.

Certified seed ensures a high-quality seed lot, and all certified seed is cleaned and labelled with information on the variety, germination rate, testing date, pure seed percentage, and inert substance percentage. Certified seed must weigh 56 pounds per bushel (about 0.72 kg L^{-1}). Seed costs do not affect production costs much. In most circumstances, quality seed will save money over cheap seed. Poor stand establishment from low-quality seed can cause yield losses and weed and disease problems (Wheat Production Handbook, 1997).

Land Preparation:

The extent of the necessary land preparation for wheat depends on the previous use of the land. Preparing the land for a dry season crop often begins in early November (e.g., India, USA), after the last rain-fed crops have been harvested. However, it is not out of the ordinary to finish land preparation before the month of October ends. When to begin the process of land preparation will depend on the crop that is currently being cultivated before the wheat crop is introduced into the cropping system. The land has to be ploughed and harrowed to a good incline. Due to the use of gravity-fed irrigation systems (e.g., India, USA) ridging is rarely necessary when growing wheat. Slope is necessary for gravity to flow water. The field's drainage can be improved by creating a gentle slope of 0.25 to 0.30 percent to a drain at the field's end. The design of the sunken beds is made so that the water can flow freely.

Seedbed Preparation:

Preparation of the seedbed differs from region to region based on factors such as the type of residue left over from the previous crop, the importance of moisture conservation, and the producer's perspective on tillage. Producing firms should aim for a firm seedbed since it improves seed-to-soil contact, which speeds up the germination process and helps plants quickly develop a stand. Even if the soils are too wet for tillage equipment, farmers will often till in an attempt to cover as much ground as possible. This causes problems with compaction and tillage pans later in the growing season. To incorporate residues, most producers either utilise a chisel operation or one or two disking's. As planting season nears, this is followed by another disking or a field cultivator. In such a farming scheme, resistant cultivars are crucial. On many extremely erodible acres, terraces, streams, and crop residue management are necessary (Wheat Production Handbook,1997).

Sowing Date:

The growth, development, and yield of crops are profoundly affected by the timing of their sowing. Wheat is a temperate crop, needing temperate conditions, thus, planting occurs in November/December with harvest occurring in March/April (e.g., India, USA). The best time to plant seeds is towards the middle of November. Wheat crop yield is affected by whether planting occurs sooner than mid-November (Falaki, 1994). It has been shown that high temperatures, aphid attacks, and stem borer infestations are all made more likely when seed sowing is delayed (Cosei, 2019).

Seed Treatment:

To ensure a successful wheat harvest, seed treatment is essential in establishing a strong crop stand from the beginning. Seed treatment guarantees a high germination rate, solid crop stand, and all else being equal, a prosperous harvest. Treating seeds with the right kind of insecticide will help reduce pest populations and promote healthier seedlings.

Seed Rate:

Wheat should be sown at a seed rate of 120-140 kg ha^{-1} up to 200 kg ha^{-1}. Broadcasting is preferred over drilling or dibbling because of the increased seeding rate it allows in large farms. To protect seeds from being eaten by ants after they have been spread, it is recommended that they be raked in after they have been scattered. (Oyewole et al., 2005).

Fertilizer Application:

Incorporating NPK fertilisers into wheat cultivation has a positive effect. The suggested application rates are 100–120 kgN ha^{-1}, 40–60 kg P_2O_5 ha^{-1}, and 40–60 kg K_2O ha^{-1} for a target yield of 5 t ha^{-1}. Seeds should be sown after P and K have been applied and incorporated into the soil. A single dose of N can be applied before planting, except on sandy soils. N should be administered in two parts, once at planting and again three to four weeks following seed sowing, on extremely permeable sandy soils (Cosei, 2019).

The second administration of nitrogen is typically suggested to be done when the ear (invisible at this time) is around 1 cm in size, according to current standards (Z31 on Zadoks scale). The Feekes and Zadoks scales are the most often used techniques for determining the developmental stages of crops. Each of these measures is based on a generally accepted approach for classifying the growth of crops at different times throughout the growing season. Identifying the crop's developmental stage is crucial for making management decisions. In instance, herbicides, fungicides, and growth regulators are often applied in the spring, during key periods of plant development (Charles, 2016).

Water Management:

Scheduling irrigation so that soil water is never less than 50% drained is critical for maximising productivity in semi-arid environments.

Under normal conditions, wheat benefits from any amount of water. Effective wheat irrigation management requires knowing the system's limits and soil moisture levels. Since most surface irrigation systems can't apply water once spring growth has commenced, autumn water applications are usually the only choice in Kansas, and in spring time in the Mediterranean area. They should result in yields on par with or greater than good dryland fallow yields.

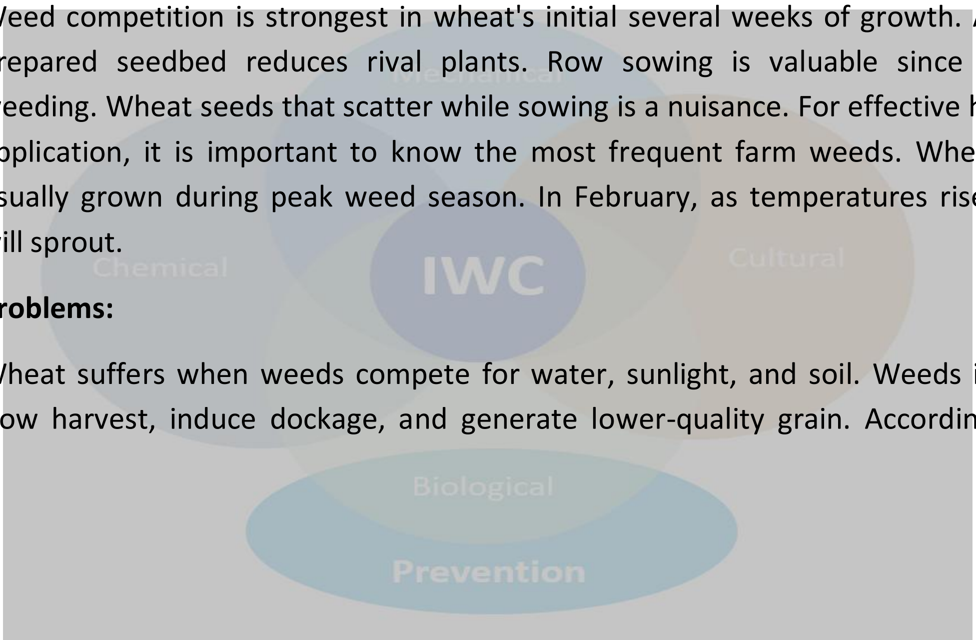
Fall sprinkler use is not advised. When dormant season ends, the soil profile can hold winter and early spring precipitation since sprinkler system operators can irrigate at any time. Wheat water use is minimal due to a modest crop and mild temperatures. So, even low-capacity irrigation systems can conserve water for a dry winter. When wheat nears jointing, keep soil moisture above 50% for the balance of the growth season. In difficult conditions, wheat needs 15 inches of irrigation (Cosei, 2019).

1.1.7 Weed and pest control:

Weed competition is strongest in wheat's initial several weeks of growth. A freshly prepared seedbed reduces rival plants. Row sowing is valuable since it eases weeding. Wheat seeds that scatter while sowing is a nuisance. For effective herbicide application, it is important to know the most frequent farm weeds. Wheat is not usually grown during peak weed season. In February, as temperatures rise, weeds will sprout.

Problems:

Wheat suffers when weeds compete for water, sunlight, and soil. Weeds in wheat slow harvest, induce dockage, and generate lower-quality grain. According to the



Weed Science Society of America, weeds cost wheat production about $50 million annually. Weed species, number, emergence timing, growing conditions, and crop state all affect how much wheat yield is lost and how difficult harvesting is. A few gigantic weeds can destroy some wheat fields, but a large population of small, late-germinating weeds may not. Weed emergence date affects weed competition and yield loss. When weeds emerge with wheat or early in the season, they have a competitive edge. Winter annual weeds affect winter wheat yields more than summer weeds. Annual weeds with a winter season grow and emerge in the fall, when wheat is planted, and generate seed in the spring.

Precautions:

Crop rotation is the best way to control these weeds. Rotating to a summer crop breaks the weeds' life cycle and allows fall and spring tillage or pesticides to limit seed formation and reduce the soil seed bank. Crop rotation will not reduce weeds until we stop them from reproducing.

By burying the seed too deeply, ploughing can diminish weed populations. Ploughing can reduce cheatgrass (*Bromus tectorum*) problems because the seed has a short lifespan and can't germinate more than 3 to 4 inches (7-10 cm) below the soil's surface. Other weeds, like jointed goat grass (*Aegilops cylindrical*), become dormant when buried, making ploughing difficult. The seed rests until next year.

Planting at different times can help control weeds. Winter annual weeds may germinate in the fall depending on soil temperature and moisture. It is better to delay wheat planting until several flushes of winter annual weeds have germinated and been eradicated by tillage or herbicides. Delaying seeding may diminish grazing and production potential, depending on weather and planting challenges.

Weed Control with Herbicides:

Herbicides can safely manage weeds in wheat. Herbicides will not solve all weed problems and should only be used in IWM programmes. Wheat weeds can also be treated with insecticides. For chemical weed control it is important to know: 1) Weed species, 2) crop and weed development stage, 3) herbicide persistence, 4) off-site migration threat. Herbicide selection requires weed identification and field weed concerns. Many weedicides/herbicides target designated weed species, and no unlisted weeds will be controlled. To get the desired results, apply herbicides at the right time. To avoid crop damage, wheat must be in the right growth stage. Early or late application reduces wheat yield. Before jointing, wheat is most resistant to post-emergence broadleaf herbicides. Depending on the herbicide, the best wheat stage changes. There are no early boot to soft dough wheat herbicides. This causes sterility, low grain fill, and low yields. Post-emergence herbicides perform best on new weeds. In the fall or early spring rosette stage, winter annual broadleaf weeds are vulnerable. Herbicide-tolerant winter annual weeds that bolted and bloomed may have destroyed wheat (Charles, 2016).

1.1.8 Harvesting, grain processing and utilization:

Harvesting:

The wheat is ready to be harvested when the weight of the grain heads begins to bend the stalks. Combine this with the wheat's golden hue, and it means it's time to harvest (10 and 12 % moisture level). Mid-November wheat plantings often yield a crop by April in Kansan; in Italy the cycle ranges fron the beginning of November to end of June. From sowing to harvest, wheat typically takes between 110 and 130 days and this range, however, is highly variable dependent on factors such as weather, seed variety, and soil quality. The land is cleared and readied for new planting after harvest. When it comes to planting wheat, farmers who follow proper crop rotation procedures wait at least three years between sowings.

Processing:

In Traditional method: Harvesting →Threshing →Winnowing → De-stoning (sorting to remove stones and foreign materials →Cleaning in water to remove adhering soils → Draining →De-hulling in mortar →Winnowing to remove bran →Washing de-hull grains →Sun-drying →Milling (Charles, 2016) .

Numerous types of wheat flour exist, each distinguished by the endosperm's hardness and the milled portion of the wheat seed. One wheat kernel contains the germ, the endosperm, and the bran, a rough outer layer. Bread made from soft wheat doesn't require as much gluten as bread made from hard wheat, hence the two are not directly comparable. The endosperm of the wheat grain is the only part of the grain that is used to make white flour. Germ flour is flour that has had the germ ground up with the rest of the grain. Flour derived from the whole grain of wheat is called "whole wheat" flour. In the process of milling flour that does not use the whole kernel, the bran and germ are removed and sold as by-products.

Utilization:

An amount of 100 g of hard red winter wheat has on average 12.6 g of protein, 1.5 g of total fat, 71 g of carbohydrates (by difference), 12.2 g of dietary fibre, and 3.2 mg of iron (17% of the daily requirement); 100 g of hard red spring wheat has 15.4 g of protein, 1.9 g of total fat, 68 g of carbohydrates (by difference), 12.2 g of dietary fibre, and 3.6 mg of iron (22% of the daily requirement) Wheat is high in starch. Wheat starch's economic value is second only to wheat glutens. Wheat flour contains gluten and starch. Flour and water can be blended into a ball of dough and separated by kneading and rinsing. Starch separates from dough and sinks, producing a gluten ball. Wheat has been a staple for millennia. Different cuisines evolved over time

(Olabanji et al., 2007). About 2/3 of the world's wheat is consumed by humans, with the rest used for animal feed and sowing (7%). Wheat starches are used to make paper sizing, laundry starch, glucose, and glucose syrup; board, adhesives, glassware, chemical carriers, ethanol, plastics, vanishes, soaps, rubber, and cosmetics (Olabanji et al., 2007).

Fig 11: some commercially available wheat products (kindpng.com)

1.2. Plant Bio-stimulants:

1.2.1. What are Plant bio-stimulants?

According to the new Regulation (EU) 2019/1009, plant bio-stimulants (PBs) are: “A plant bio-stimulant shall be an EU fertilising product the function of which is to stimulate plant nutrition and processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: i) nutrient use efficiency, ii) tolerance to abiotic stress, iii) quality traits, or iv) availability of confined nutrients in the soil or rhizosphere” (EU, 2019).

For the first time in the scientific literature, Kauffman et al. (2007) defined bio-stimulants as "materials, other than fertilisers, that encourage plant development when applied in modest quantities" with certain revisions.

Du Jardin (2015) defined PBs as follows: “A plant bio-stimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content”.

It is generally observed that signalling bioactive molecules in the primary and secondary metabolisms of plants are responsible for the increased germination, seedling growth, and crop productivity that follow the application of PBs (Calvo et al., 2014).

The agricultural industry is currently confronted with the simultaneous difficulties of increasing production to feed a growing global population, maximising the effectiveness of resource usage, and minimising damage to ecosystems and human health. Natural plant bio-stimulants (PBs) have the potential to replace toxic pesticides and fertilisers as a result of their ability to boost blooming, plant growth, fruit set, crop yield, and nutrient utilisation efficiency (NUE) (Colla and Rouphael, 2015).

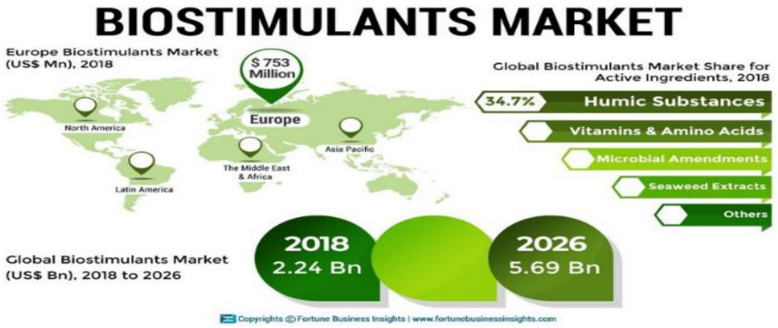


Fig 12: Plant bio-stimulants (PBs) global market

The global market for bio-stimulants was $2,241 million in 2018, with a CAGR of 12.5% from 2013 to 2018 (Calvo et al., 2014). In the EU, bio-stimulants are worth 200 to 400 million euros (with a 10% annual CAGR) (EBIC). Bio-stimulant's impacts on plant growth, stress reduction, and disease prevention improve plant output, yield, and quality.

Properties: Bio-stimulants are natural chemicals made of organic and inorganic elements, and most components are unknown. Isolation and research of a single component are nearly difficult, and a bio-stimulant's success is likely owing to the synergistic activities multiple bioactive compounds. They contain humic and fulvic acids, protein hydrolysates, nitrogen compounds, seaweed extracts, helpful fungi, and bacteria. Plant bio-stimulants include fungi and bacteria that affect the species composition of soil or plant organisms. Their presence may speed up degradation or restrict fungal and bacterial growth (Battacharyya et al., 2015). Popular bio-stimulants include *Glomus intraradices*, *Trichoderma atroviride*, *Trichoderma reesei*, and *Heteroconium chaetospira* (du Jardin, 2015). Useful bacteria include *Arthrobacter*, *Enterobacter*, *Acinetobacter*, *Pseudomonas*, *Ochrobactrum*, *Bacillus*, and *Rhodococcus*. Biostimulants can increase photosynthetic activity and derived compounds or accelerate secondary metabolism by stimulating biosynthetic pathways.

Benefits: Food waste or agro-industrial by-products can be used to make bio-stimulants. Using by-products as source material for bio-stimulants is part of the circular economy approach for sustainable agriculture. Developing bio-stimulants from by-products reduces waste and benefits agriculture, the food business, registration and distribution companies, and consumers. Absence/reduced pesticide residue, low collection and storage costs, sufficient supply, and synergy with other valorisation paths are criteria for selecting bio-stimulant by-products. NOSHAN, SUNNIVA, and Bio2Bio are national and international initiatives that examine the vaporization of by-products for the food and agriculture industries and the mechanism of action of bio-stimulants from organic waste streams. Several waste-derived bio-stimulants are beneficial in agriculture and horticulture, including vermicompost, composted urban waste, sewage sludge, protein hydrolysate, and chitin/chitosan derivatives. As the global market for bio-stimulants grows, more research and development will broaden the list of by-products. Due to global nutrient imbalance, bio-stimulants must be marketed (Xu and Geelen, 2018).

- Diverse physiological functions: Physiological roles include photo-protection and lateral root formation. Cellular systems assist functions, including antioxidants scavenging reactive oxygen or enhanced auxin transporter synthesis. Physiological activities and cellular mechanisms are bio-stimulants "modes of action'. These routes of action explain bio-stimulants' agricultural activities, such as greater tolerance to abiotic stress (oxidative stress) or increased N use efficiency (which depends on the foraging capacity of roots, hence on lateral root density). Agricultural functions can convert into economic and environmental benefits: higher crop production, fertiliser savings, improved crop quality and profitability, enhanced ecosystem services, etc (du Jardin, 2015).
- All bio-stimulants improve nutrition efficiency, abiotic stress tolerance, and/or crop quality attributes, according to research. Quality features include nutrition, protein content, shelf life, etc. Bio-stimulants should be defined by these convergent actions

and several bio-stimulants stimulate pathogen response by elicitors and plant gene regulators (chitosan, laminarin, some PGPRs, etc.). Growing consensus among regulators and stakeholders to maintain bio-stimulation and biocontrol separate regulatory-wise (du Jardin, 2015).

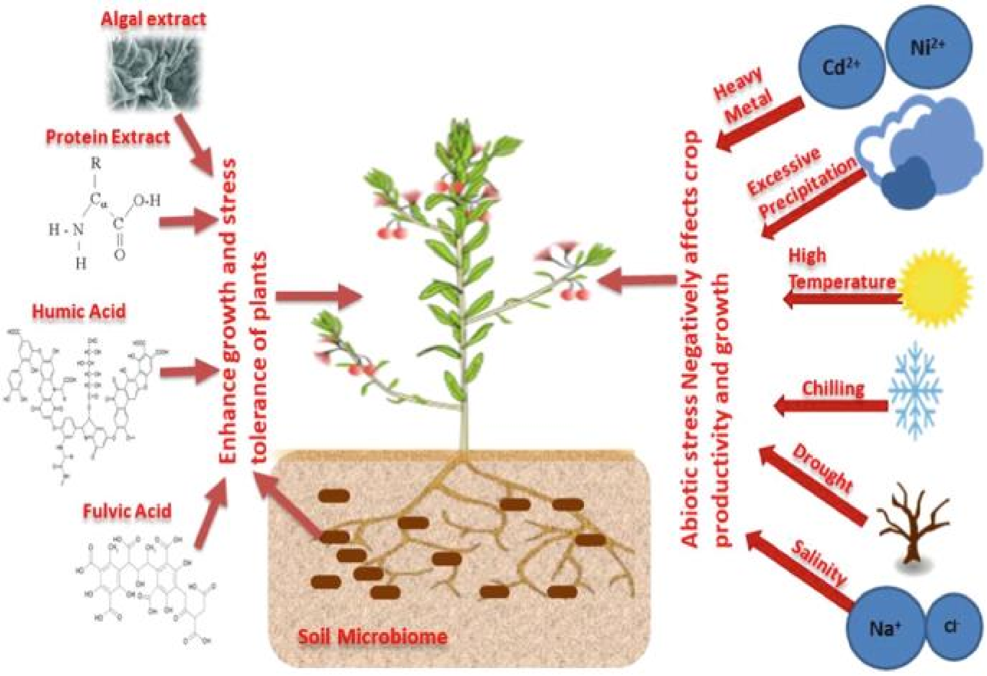


Fig 13: Role of plant bio-stimulants (PBs) (Pandey et al., 2022)

1.2.2. Composition of Bio-stimulants:

Bio-stimulants come in ready-to-use extracts or powder for aqueous solutions that influence the root structure, which increases its nutrient absorption. Foliar extracts prevent biotic and abiotic stress. Plant circadian rhythms must be considered and best to apply in the morning when stomata are open, and absorption is high. They can be applied as soil preparations (powders, granules, or solutions) or liquid foliar applications [41]. Bio-stimulants containing humic and nitrogen compounds are generally administered directly to the soil, while plant and seaweed extracts are utilised foliar. Bio-stimulants can be added to irrigation water and absorbed by plants (e.g., Kelpak SL (*Ecklonia maxima* extract) was sprayed in *Phaseolus vulgaris* L. during the vegetative stage).

Seaweed biomass or meal can be utilised as a bio-stimulant, although this method has some limits. Biomass and meal may be used near the source of seaweed due to transit issues and added to the soil before planting to enhance the substrate. Agro-technical methods like ploughing mix biomass or meal with topsoil. (Drobek et al., 2019)

1.2.3. Types of Bio-stimulants:

Scientists, regulators, and stakeholders all agree on a core set of PBs categories that include both chemicals and microbes (Calvo et al., 2014; du Jardin, 2012 ; Halpern et al., 2015).

Humic Acids And Fulvic Acids: Humic (alkali-soluble and have high molecular weight) and fulvic acids (alkali- and acid-soluble and have lower molecular weight).

Humic compounds are organic materials derived by microbial metabolism and chemical and biological modification of plant and animal detritus (Canellas et al., 2015). The major component of humic substances is humus, a brown to black carbon composite. Humus is created through the controlled fermentation of plant and animal detritus by soil bacteria (Canellas and Olivares, 2014).

Natural decomposition of plant and animal matter produces humic and fulvic acids as the last by-products. Humic acids are a rich carbon source that feeds beneficial bacteria and helps them flourish. Humic bio-stimulants improve germination and seedling growth and boost tolerance to abiotic stressors. (Trevisan et al., 2010).

Humic compounds are made up of humic acids that dissolve only in alkaline conditions, and fulvic acids that dissolve in acid and alkaline environments. Unlike fulvic acids, humic acids have bigger molecules that are 100,000 times larger than fulvic acids. Humic and fulvic acids boost soil and plant health. They affect plant root (particularly hairy root) and shoot growth and boost plant strength under biotic (diseases and pests) and abiotic (salinity, drought, frost, heat, etc.) stress and help plants absorb nutrients. Particularly helpful for plants is fulvic acid chelated with iron, copper, manganese, and zinc. During the early phases of plant development, humic and fulvic acids are utilised to increase the organic matter content of the soil and encourage root formation. Humic and fulvic acid concentration vary by source. Leonardite, an immature form of lignite, produces soluble, alkaline compounds with high humic acid and low fulvic acid contents. Herbal lignin products are acidic with high fulvic acid and low humic acid.

Protein hydrolysates (PHs): Plant bio-stimulants called protein hydrolysates (PHs) are "mixtures of polypeptides, oligopeptides and amino acids that are generated from protein sources by partial hydrolysis" (Schaafsma, 2009). They contain peptides and free amino acids, but also carbohydrates, minerals, phenols, and phytohormones (Calvo et al., 2014; Ertani et al., 2014). The use of plant- and animal-derived (e.g., legumes, alfalfa hay, maize wet-milling, and vegetable by-products) protein hydrolysates in agriculture improves plant growth and crop performance and may reduce the use of mineral fertilisers (Apone et al., 2010). Animal-derived PHs may infect plants with bioactive peptides and viruses, endangering human health, so that they should be autoclaved at 0.3 MPa and 134 °C to denature bioactive peptides and viruses (Taylor 2000; Ertani et al., 2013; Colla et al., 2015). PHs stimulate seed germination and seedling growth by acting like hormones (auxin-like and gibberellin-like activity) (Colla et al., 2015).

PHs increase shoot and root biomass and productivity in horticultural crops (Colla et al., 2014, Ertani et al., 2014).PHs applied to plant leaves and roots boost Fe and N metabolism, nutrient absorption, and water and nutrient utilisation efficiency for macro and microelements (Cerdán et al., 2009; Halpern et al., 2015) (Colla et al., 2015).

Seaweed extracts: Green, brown, and red macroalgae are seaweeds. Brown seaweed extracts are commonly used in horticulture crops to promote plant development and improve crop tolerance to abiotic challenges such salinity, severe temperatures, nutrient deficiencies, and drought. Seaweed extract contains polysaccharides, fatty acids, vitamins, phytohormones, and minerals (Battacharyya et al., 2015).

Based on quantity and distribution, brown seaweeds (*Phaeophyta*) are utilised to make agricultural and horticultural extracts. *Ascophyllum nodosum*, *Ecklonia maxima*, *Macrocystis pyrifera*, and *Durvillea potatorum* are commercially used brown seaweeds (Khan et al., 2009). The chemical makeup of extract relies on the extraction procedure and the chemicals utilised. So, extracts of the same seaweed raw material prepared by different extraction techniques may have varying biological activity (Kim, 2012; Khairy and El-Shafay, 2013).

Seaweed extracts are commonly used on horticultural crops as liquid extracts or soluble powder. Mix liquid extracts with irrigation water and drip-irrigated crops to apply near the plant's root and effectiveness depends on plant growth stage. Dwelle and Hurley (1984) discovered that seaweed extract increased potato output when applied 2 weeks after tuber start (Battacharyya et al., 2015).

Phosphite (Phi): Phosphite (Phi) is a new horticultural bio-stimulant. Experimental research shows that Phi can act as a biocide and influence plant production and productivity. Phi's positive effects on plant metabolism are more apparent when given to the roots in hydroponic systems or to the leaves as foliar sprays. Phi is a potent insecticide against pathogenic bacteria and *Oomycetes*. Phi as a sole plant P-source is still debatable. Microorganisms oxidise Phi when it is added to soil. Thus, Phi can become a P nutrient after microbial oxidative processes. Transgenic plants with microbial genes that allow them to utilise Phi as a sole P-source have opened up new uses for this P-containing molecule. Phi has shown its usefulness against numerous stress factors and enhanced crop yield and quality. Molecular, biochemical, and physiological advances have validated Phi's function in horticultural output and quality. Although Phi absorption, transport, and subcellular localization have made progress, a deeper knowledge of Phi's effects on plant metabolism is still needed (Gómez-Merino and Trejo-Téllez, 2015).

Chitosan: Deacetylated chitin is a biopolymer found in fungal cell walls, insect exoskeletons, and crustacean shells. Chitosan impacts plant responses based on structure and concentration.

Chitosan inhibits microbial growth and membrane integrity (Xu et al., 2007, Palma-Guerrero et al., 2008), lowering disease incidence and severity in numerous crops.

Chitin is characterised from chitosan by the larger proportion of N-acetyl-D-glucosamine over D-glucosamine in the polymer chain, with more than 95% N-acetyl-D-glucosamine and less than 5% D-glucosamine in crab shells, shrimp shells, and squid pens (Rinaudo, 2006, Sagheer et al., 2009). Based on the organisation of the chitin polymer in its native state, two major forms of chitin may be observed: -chitin in shrimp shells and -chitin in squid pens (Rinaudo, 2006) (Xu and Geelen, 2018).

Silicon Bio-stimulant: Despite being the second most prevalent element in the crust of the earth, [silicon Si] is not thought to be crucial for plant nourishment. But evidence from both theory and practice supports the positive effects of silicon on the growth and development of many plant species, particularly when they are subjected to biotic or abiotic stress. Si is mostly found as monomeric silicic acid [H_4SiO_4] in soil solution at values ranging from 0.01 to 2.0 Mm. Because H_4SiO_4 does not dissociate at pH values lower than 9, plants can either actively or passively uptake Si in this non-ionic state, depending on the external concentration of Si in this non-ionic state, depending on the external concentration of silicon and their own needs. Mostly in the form of a polymer of hydrated amorphous silica, Si accumulates in a variety of tissues.

The use of silica as a bio stimulant in horticulture can be expanded currently. Si is used in several commercial crops to increase resistance to abiotic stressors, diseases, and infections. Si reduces the effects of salts, drought and nutritional stress as well as climatic stress, reduces the toxicity of metals and metalloids, and may delay plant sencensce process.

Key mechanisms:

Silica deposition inside plant tissues, provide mechanical stability and uprightness and controls nutrition and water mobility within the plants.

Activation of plant antioxidants systems.

Composition or co-precipitation of harmful metals with silicon in soil and plant tissues.

Modulation of gene expression and signalling by phytohormones, although currently, lack of evidence that there is direct involvement in Si metabolic activity.

2. Aim of the thesis :

As the Agriculture has been the most prominent sector in feeding the world population. To meet the demands of these increasing population, scientists have been continuously developing the innovations to enhance the sustainability of agriculture production systems through a significant reduction of pesticides and fertilizers.

A Favourable and environmentally friendly innovation would be use of natural bio-stimulants that enhance flowering, plant growth, fruit set, crop productivity and nutrient use efficiency.

The Aim of this study was to evaluate the efficiency of new bio-stimulants extracted from rice husk and sage, that were experimented on three wheat varieties [Bologna, Vivendo and Solehio] by spraying it at flowering stage and its effects were compared with untreated controls and two benchmark commercial bio-stimulants obtained from protein hydrolysates'. The aim of this study was to determine the influence of the new bio-stimulant and its role in improving the plant metabolism and increasing the vegetational, yield and quality parameters. This experiment would also benefit in identifying the key physiological, biological traits useful for future studies and the developments in bio-stimulants.

3.Materials and Methods:

3.1 Field Trail Setup : The trial was conducted at the "Lucio Toniolo" experimental farm of the University of Padua located at municipality of Legnaro, Padua (NE Italy) during the 2020-2021 growing season. The soil was silty loam (fulvi-calcaric-cambisol]; According to USDA classification the chemical -physical properties of the experimented land was:

Table 1. Main chemical and physical properties of the land of 'Lucio Toniolo'

Property	Value
Silt %	60
Clay %	20
Sand %	20
PH	8.0
Organic matter %	1.7
CEC	11.4 $\mathrm{cmol^{+}\ kg^{-1}}$
N	1.1 $\mathrm{g\ kg^{-1}}$

The experimental set-up consisted of a completely randomized block design with three replicates (n = 3); each plot measured 6 x 4 m (24-m2) and included 24 wheat rows, 13 cm apart.

3.2 Crop Management:

Climatic conditions and soil water availability:

Meteorological parameters:

Meteorological data on air temperature and rainfall were collected from ARPAV "Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto" database (https://www.arpa.veneto.it/arpavinforma/bollettini), from the Legnaro station, placed within the experimental farm. Monthly temperature and monthly average precipitation during the wheat growing trail period season [November-June] compared with historical 10-year mean were analysed.

Soil moisture. Three soil-core samples (4 cm ø and 25 cm-depth in the soil) were taken from each plot/variety, from Bologna, Vivendo and Soheio varieties (3 replicates). Soil was collected in plastic bags in order to avoid humidity loss and transported to the laboratories of “L. Toniolo” experimental farm of the University of Padova. Here, soil samples were weighed, oven-dried at 105 °C for 48 hours, and then weighed again, to determine the % of humidity.

Before wheat sowing, the soil was ploughed to a depth of 0.3 m, incorporating the residues of the previous crop (sugar beet), and harrowed twice at 0.2 m.

On 30 October 2020 - The soil was fertilized in pre-sowing with a ternary fertilizer NPK 8-24-24 at a dose of 400 kg ha^{-1}, corresponding to 32 kg ha^{-1} of nitrogen (N), 96 kg ha^{-1} of P_2O_5 (phosphorus) and K_2O (potassium).

On 18 February 2021 - The nitrogen cover fertilizations were carried out in the tillering phase by applying 54 kg N ha^{-1} as ammonium nitrate (rate 27% N),

On 7 April 2021 - During the stem elongation phase 87 kg N ha^{-1} was applied as urea (rate 46% N).
No foliar fertilization was performed in the earing phase, in anticipation of the application of the bio-stimulating product.

The overall dose of nitrogen supplied to the wheat was therefore 173 kg ha^{-1}.

The phytosanitary control was performed as follows:

31 March 2021: fungicide treatment with BLAISE ULTRA (dose 1 L ha^{-1}; a.i. azoxystrobin + tebuconazole) + herbicide treatment with GRANSTAR ULTRA SX (dose 50 g ha^{-1}; a.i. tifensulfuron methyl + tribenuron methyl) + adhesive VECTOR (dose 0.6 L ha^{-1}; ethoxylated isodecyl alcohol).

08 May 2021: fungicide treatment with AMISTAR (a.a. azoxystrobin; dose 0.5 L ha-1) + CARAMBA (a.a. metconazole; dose 1 L ha-1).

28/05/2021: insecticide against brown marmorated stink bug (*Halyomorpha halys*) with EPIK SL (dose 1 L ha^{-1}; a.i. acetamiprid).

Bio-stimulants used for trail:
The 3 wheat varieties used for this trail were “Bologna” (SIS, Bologna, Italy), chosen for his high bread-making quality and one of the most widespread in Northern Italy, “Vivendo” (RAGT, Ferrara, Italy) and “Solehio” (ISTA, Potenza). Sowing took place on 4 November 2020 and harvesting on 28 June 2021.

Four treatments were compared in this experiment:

- UNTREATED CONTROL
- NEW BIO-STIMULANT (dose 3 kg ha^{-1})
- BENCH-1 (dosage 3 kg ha^{-1})
- BENCH-2 (dosage 3 kg ha^{-1})

Fig.14: NEW-BIO vs two commercial benchmarks BENCH-1 and BENCH-2 in liquid formulation

The bio-stimulant formulation “NEW-BIO” is the innovative product being evaluated in this thesis. It is a bio-stimulant composed of rice husk extracts (with a high content of linoleic acid) and sage extracts, which preliminary tests, showed positive agronomic effects in soft wheat var. Bologna. This bio stimulant was evaluated in comparison to two commercial benchmarks, and to an untreated control.

The three bio-stimulants were applied at a dose of 3 kg ha^{-1} by foliar application (Fig. 15) in the full flowering phase (BBCH 65 phase), using a backpack pump spraying a volume of 500 L ha^{-1} (concentration of the bio-stimulant 0.6%) on 20 May 2021 on var. Bologna, and 26 May 2021 on var. Vivendo and Solehio. Following heavy rains and high winds in the days after the application of the products the wheat suffered partial lodging (Fig. 16].

Fig 15: Bio-stimulants application at flowering stage of wheat

Fig 16: Wheat lodging due to wind breaks.

Harvesting wheat crop (Bologna variety):

The harvest of Bologna variety took place through a plot combine harvester on 28 June 2021 (Figure 17).

Fig 17: Harvesting of wheat 'bologna' variety using a combine harvester

Due to the lodging problems that affected var. Bologna a few days after the application of the bio-stimulant products, it was decided to carry out a parallel comparison between the bio-stimulant products also on two further varieties of common wheat: Vivendo (RAGT, Ferrara) and Solehio (ISTA, Potenza) cultivated in mini-plots of 2 m^2. The first has a high protein content variety and is classified as a superior bread making wheat, while the second one has a medium protein content and is identified as a bread making wheat.

Comparison between treatments was performed using a fully randomized block trial design with three replicates per treatment (n = 3). The application of the bio-stimulants took place on 26 May 2021 using a backpack pump with a spraying volume of 500 L ha^{-1} (Figure 18). Harvesting of these two varieties, which did not suffer lodging, was made manually.

Fig 18: Foliar application through a backpack pump of bio-stimulant products on mini plots of 2 m^2 in the Vivendo and Solehio wheat varieties.

3.3 Plant Analysis : During experimentation the parameters detected were

Vegetative parameters:

In the wheat field and in mini-plots, the normalized difference vegetation index (NDVI) areas was measured by a Greenseeker (Ntech Industries, CA-USA) directly on the crop canopy, while plants chlorophyll content was estimated with SPAD-502 chlorophyll meter on plants collected and analysed at the experimental farm "Lucio Toniolo" in Legnaro.

NDVI - Normalized Difference Vegetation Index:

The normalized difference vegetation index (NDVI) was used to assess the greenness of wheat canopy and is calculated as the difference between near-infrared (NIR) and red (RED) reflectance (Ref) divided by their summation:

NVDI= (RefNIR-RefRED) / (RefNIR+RefRED)

NDVI ranges between 0 to +1. A higher value of NDVI reflects healthy vegetation with a high density of green vegetation. For this experiment, we used a Greenseeker (Ntech Industries, CA-USA) (Figure 19) that is a handheld device with a sensor that records 10 readings per second. To make the measures, the sensor must be kept parallel to the ground at an optimal distance from the crop canopy ranging from 30 to 40 cm.

Green index and ground cover degree through weekly measurement of the NDVI (Normalizer Difference Vegetation Index) from the end of the flowering phase (post application) and until maturation-senescence.

Fig 19: NDVI measurement.

SPAD - Soil Plant Analysis Development:

To estimate the chlorophyll content of wheat leaves we used the handheld chlorophyll meter SPAD-502 (Minolta Corporation, Ltda., Osaka, Japan) (Figure 10). This equipment measures the transmittance of red light (650 nm) and infrared light (940 nm) through the leaf and gives out a SPAD (Soil Plant Analysis Development) value, ranging from 0 to 100, that is attributable to the amount of chlorophyll present in the leaf tissues (Minolta, 1989). The main advantages of this method are: (i) light weight of the equipment, (ii) the analysis can be made without the destruction of the leaves, (iii) simple method, (iv) a great number of leaf samples can be measured and recorded in a short period.

Leaf chlorophyll content by measuring the SPAD index (Soil Plant Analysis Development) was performed simultaneously with the NDVI measurement of 6 plants for parcel, on Vivendo and Solehio varieties.

Fig 20: SPAD measurement on wheat leaves.

3.4 Productivity and Grain quality parameters:

Yield: The total weight of the samples (stems and grains) was determined, and the total number of spikes was counted. Spikes were then cut and threshed using a stationary combine harvester. Grain and straw of each sample were weighed in order to determine yield and the Harvest Index (HI).

Testing weight: The testing weight is a ratio between the weight and the volume of the grains. It is an important indication of the flour yield of wheat. Testing weight varies according to the shape, thickness, roughness, and humidity of grains. Thus, variety choice, sowing season, and environmental conditions can also have significant effects on the testing weight. The determination of the testing weight was made using the GAC 500XT.

Fig 21: GAC 500XT for grain testing weight measurement.

Thousand Grain Weight – TGW Thousand grain weight is related to the size (varietal trait) and extent of grain filling. This value was determined by weighing three repetitions of 100 seeds from each of the 3 varieties, then averaged and multiplied per ten, in order to obtain the TGW.

Quality parameters: The quality parameters of wheat grains were assessed by NIRS (Near InfraRed Spectroscopy) technology. The samples harvested on 28 June 2021 were analysed using Infratec-1241 equipment. This method is based on the interaction between the grain matter and infrared radiation. When an object is exposed to a light beam, part of the radiation is absorbed, and the remaining part is transmitted or reflected. The amount of light absorbed is strictly related with the chemical composition of the analysed material. To obtain more information about the sample, different wavelengths are applied. In our study, we used the Infratec-1241 to determine protein content, percentage of humidity, percentage of humid gluten, and Zeleny index. The Zeleny index predicts the overall quality of gluten and therefore the baking quality of wheat.

Fig 22: NIRS (Near InfraRed Spectroscopy)

3.5 Statistics analysis:

The data from all the assessed parameters were subjected to ANOVA within Stat graphics Centurion XI software (Adalta, Arezzo, Italy). Separation of means was set at $P \leq 0.05$ with the Newman–Keuls test.

4.Results and Discussion:

Climatic conditions during the trial:

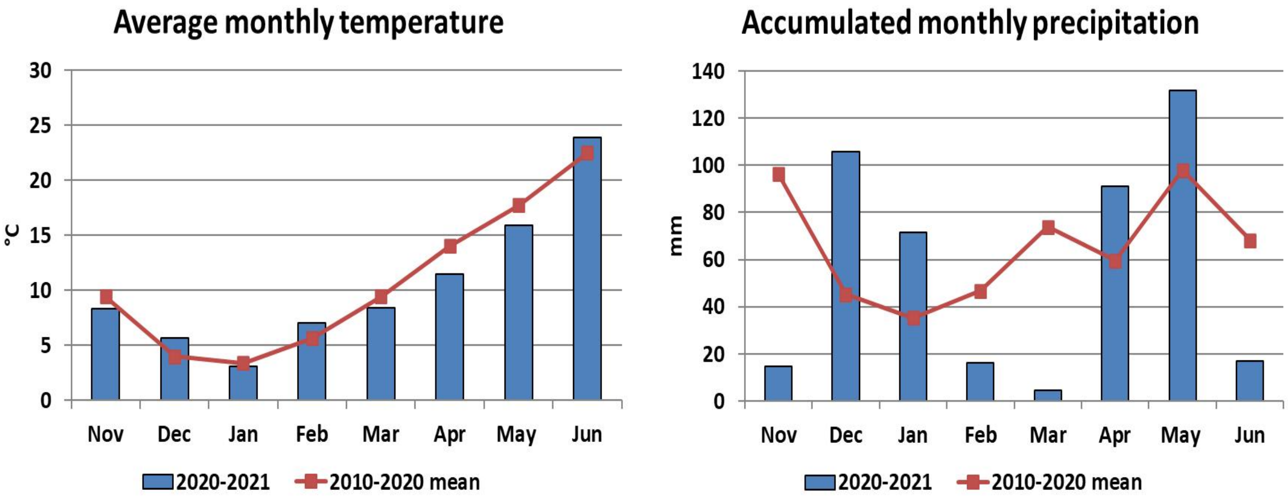


Fig 23: Average monthly temperature and accumulated monthly precipitation at L. Toniolo Experimental Farm (ARPAV meteorological station).

During the study period, the monthly average temperature recorded from November 2020 to June 2021 was almost similar with the last ten-year historical mean, although the major difference observed during 2020-2021 season was the temperature increase in the months of December 2020, February 2021, and June 2021 with almost ~+2°C compared to the historical data.

With regard to the 2020-21 monthly precipitation, significant differences have been measured compared with historical data, the rainfall was much higher in the months of Dec. 20 [100 mm vs. 48 mm], January 21 [70 mm vs. 39 mm], April 21 and May 21 than the past years .Contrarily, the precipitation in the months of November 20, Feb 21, March 21, and June 21 were very low (<20 mm).

4.1.Vegetative parameters:

4.1.1.NDVI:

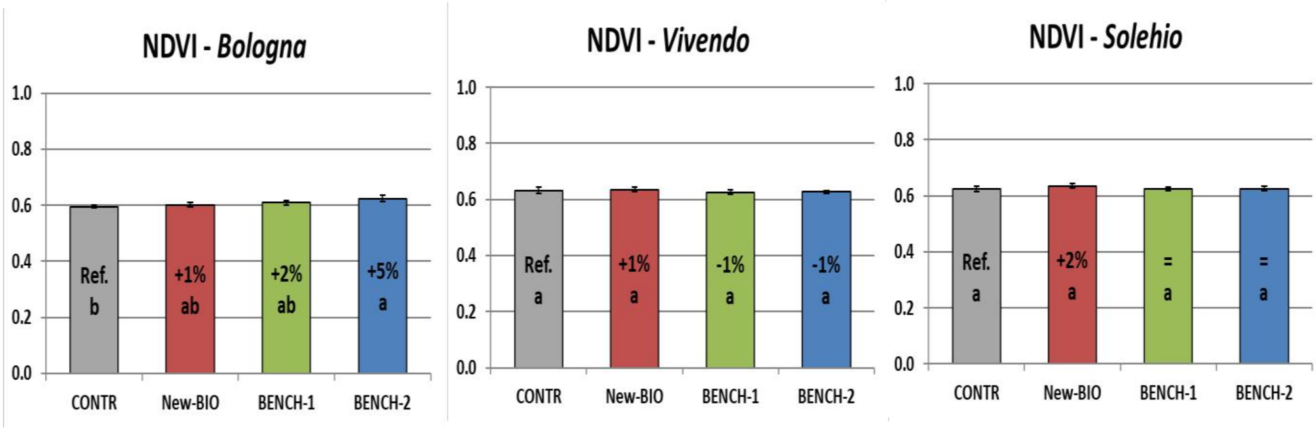


Fig 24: Average (from earing to maturity) of Normalized Difference Vegetation Index (NDVI) in different treatments. Percentages inside histograms indicate the variation of each treatment vs. untreated controls. Letters indicate significant differences between treatments within each variety (Student-Newman-Keuls test; P≤0.05).

The average (from earing to maturity) NDVI values were compared for all the three varieties to untreated controls. The average NDVI value for greenness in Bologna variety consists of high NDVI value in Bench-2 treatment [>0.6] with the variability of +5 % compared to untreated control.

Vivendo variety performed higher NDVI value for the new bio stimulant, but with an increase of +1% [>0.6] compared to untreated control.

In Solehio variety, the average NDVI value compared with untreated controls, Bench 1 and Bench 2 were similar [0.6] and for New bio-stimulant [>0.6] it was higher with +2 %.

It concludes that all the three varieties performed higher canopy greenness with slight differences.

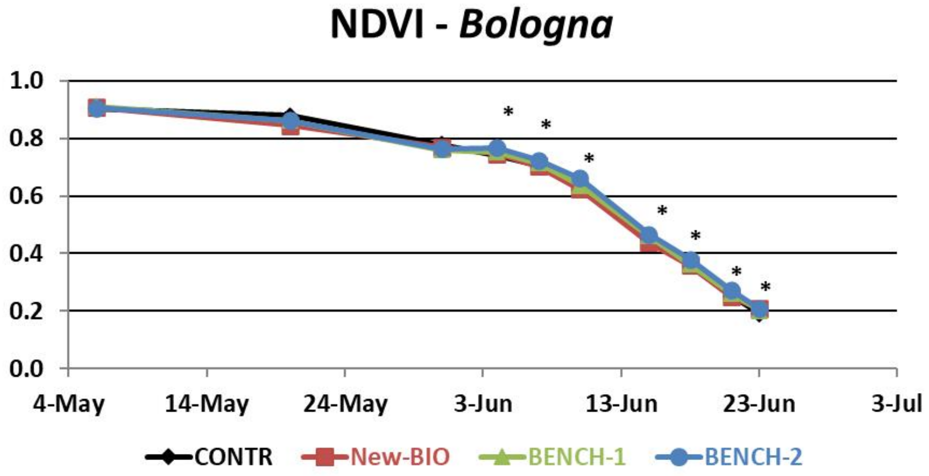


Fig 25: Dynamics of Normalized Difference Vegetation Index (NDVI) among the treatments from earing to maturity of Bologna wheat variety. Asterisks indicate significant differences among treatments for each date (Student-Newman-Keuls test; P≤0.05).

In Bologna variety, the initial NDVI Value when taken on 4 May were similar for all the treatments as well as on 14 May. It is observed that there is a little increase in control treatment when compared with the other 3 treatments. After 24 May 2021, it is observed that there is an increase in Benchmark 2 bio-stimulant treatment when compared with the other three treatments. Later, NDVI values were reduced in all the 4 treatments after 13 June to harvest.

4.1.2.SPAD:

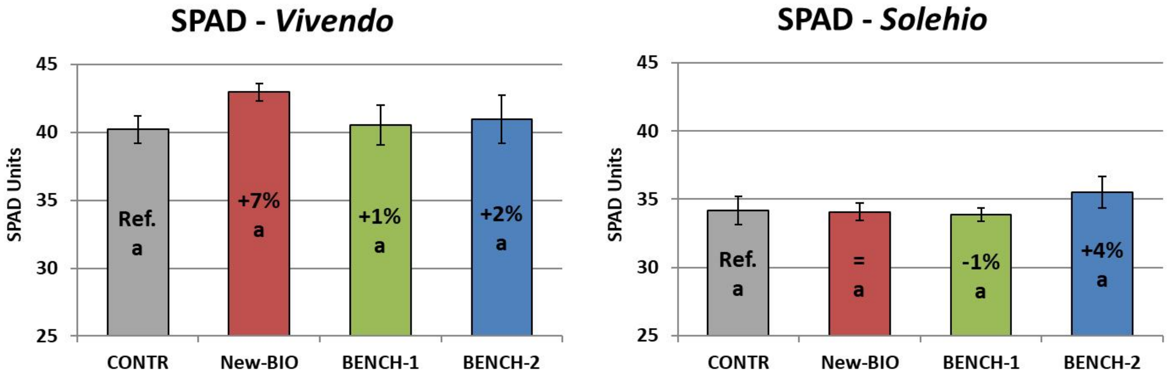


Fig 26: N. Average (from earing to maturity) chlorophyll content (as SPAD units) in the flag leaf of two wheat varieties. Percentages inside histograms indicate the variation of each treatment vs. untreated controls. Letters indicate significant differences between treatments within each variety (Student-Newman-Keuls test; *P*≤0.05).

Because of lodging in var. Bologna, SPAD measurements were only taken for Vivendo and Solehio varieties. The average chlorophyll content in the flag leaf of 2 wheat varieties were measured and the significant difference observed in Vivendo with high SPAD value raising to +7% in the New bio-stimulant [43 units], while the Bench 1 treatment had +1 % increase and the Bench 2 had +2 % increase compared to controls.

In Solehio variety, the average SPAD values for the new bio-stimulant were equal with controls, while Bench 1 is observed with the significant difference of -1 % compared to controls and Bench 2 is showing +4% increase.

When both varieties were compared the majority of chlorophyll content is seen for Vivendo variety having higher values in all the treatments when compared with Solehio variety.

So, in general the variability for chlorophyll content and the values in the both the varieties were ranging from -1 % to +7 %.

4.1.3.YIELD:

Grain yield:

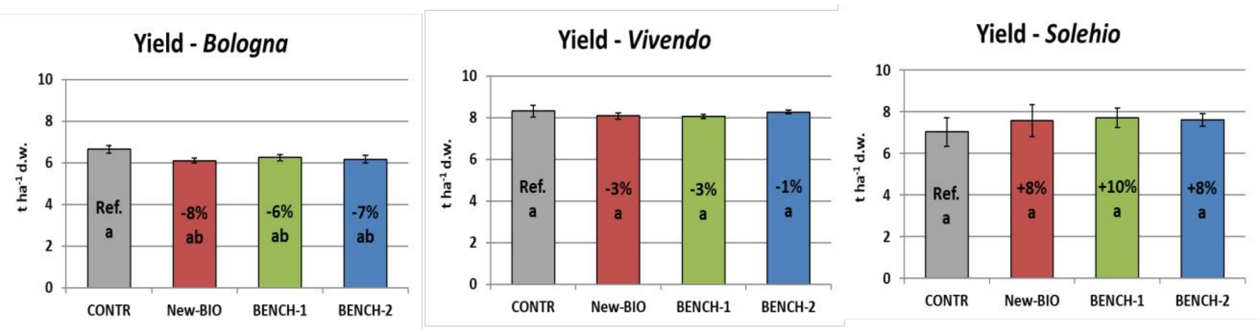


Fig 27: Grain yield (as t ha^{-1} d.w.) of the three wheat varieties. Percentages inside histograms indicate the variation of each treatment vs. untreated controls. Letters indicate significant differences between treatments within each variety (Student-Newman-Keuls test; $P \leq 0.05$).

The grain yield was measured for three varieties Bologna, Vivendo and Solehio after harvest. The yield in Bologna variety was lower when compared with the other 2 Var. Vivendo and Solehio. In Bologna, the yield with the New bio-stimulant, Bench 1 and Bench 2 treatments was observed with -8%, -6%, -7%, respectively, when compared with the untreated control with 6.5 t ha^{-1}.

In Vivendo, the yield the untreated control registered 8.4 t ha^{-1}. The treatments like the new bio-stimulants, Bench 1 and Bench 2 had the significant difference of -3 % ,-3% and -1% decrease compared to the untreated control.

In Solehio, the yield was higher in Bench 1 treatment having 7.8 t ha^{-1} compared to the untreated treatment [7 t ha^{-1}] and the yield content for the Bench 2 treatment and New bio-stimulants were having both +8%.

So, in general the yield in Vivendo was higher with 8.3 t ha^{-1} compared to other varieties. All the bio stimulants enhanced a bit grain yield in var. Vivendo.

4.1.4.Testing weight:

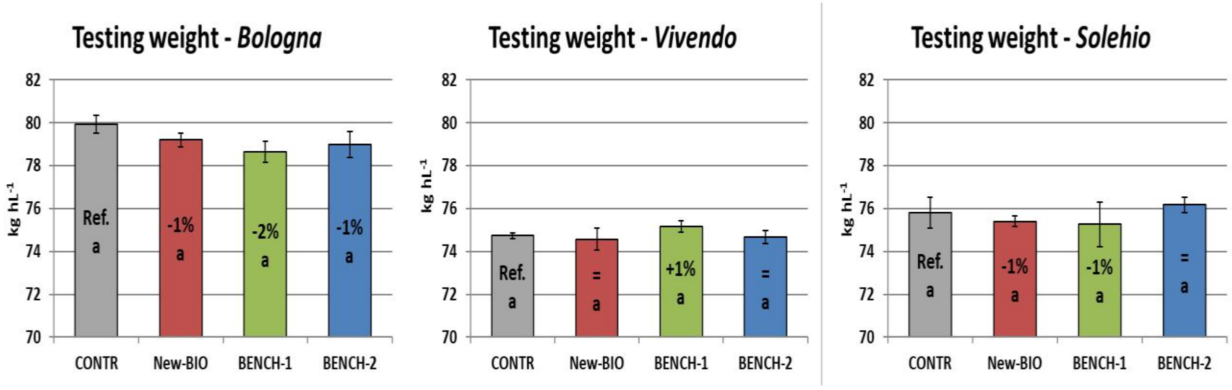


Fig 28: Testing weight (as kg hL^{-1}) of the three wheat varieties. Percentages inside histograms indicate the variation of each treatment vs. untreated controls. Letters indicate significant differences within each variety (Student-Newman-Keuls test; $P \leq 0.05$).

The testing weight is a ratio between the weight and the volume of the grains. It is an important indicator of the flour yield of wheat.

Testing weight is compared for all the three varieties and the Bologna variety is having higher Testing weight with 79 kg hL^{-1} compared to the other 2 varieties. The high testing weight was observed in the untreated control of Bologna variety, while the new bio-stimulant, Bench 1 and Bench 2 are registered with small reductions of -1%, -2%, -1% respectively.

In Vivendo variety, Bench 1 treatment is showing higher value with +1 % when compared with the control treatment; the other 2 treatments new Bio-stimulant and Bench 2 are similar to the control.

In Solehio, the untreated control and Bench 2 were similar, while the new bio-stimulant and bench 1 treatment are registered with -1 % decrease compared to the control.

4.1.5.Harvest index:

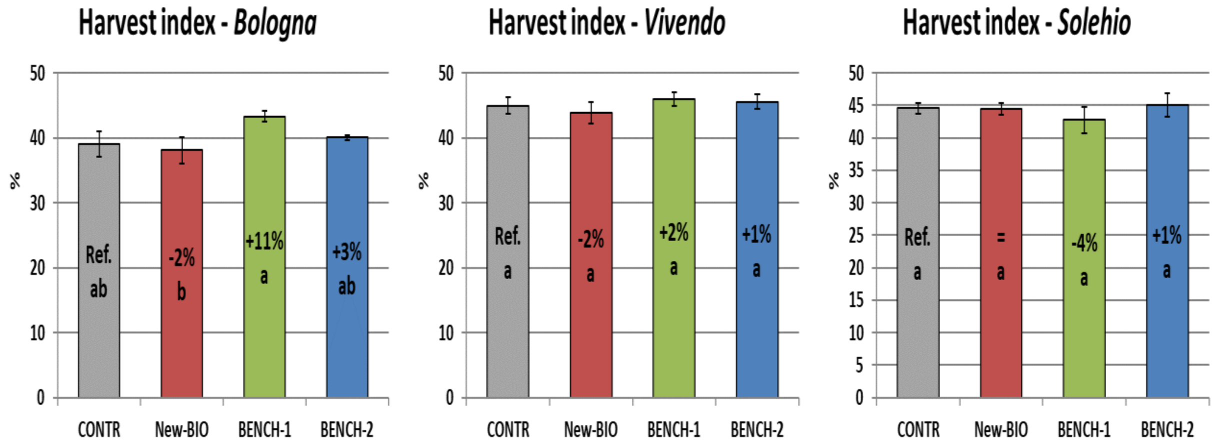


Fig 29: Harvest index of the three wheat varieties. Percentages inside histograms indicate the variation of each treatment vs. untreated controls. Letters indicate significant differences between treatments for each date (Student-Newman-Keuls test; *P*≤0.05).

Harvest Index was recorded for the three var. Bologna, Vivendo and Solehio. In these varieties the highest harvest index is observed for Vivendo and Solehio. In Vivendo, Bench 1 treatment is registered as high harvest index with +2% [47 %] compared with untreated treatment and the other Bench 2 and new bio-stimulants accumulated with -1% and -2% harvest index, respectively. In Solehio variety, the control treatment and the new bio-stimulants were similar, and Bench 1 is observed with -4 % decrease and +1 % increase in Bench 2 treatment compared to the control.

4.1.6.Thousand Seed Weight (TSW):

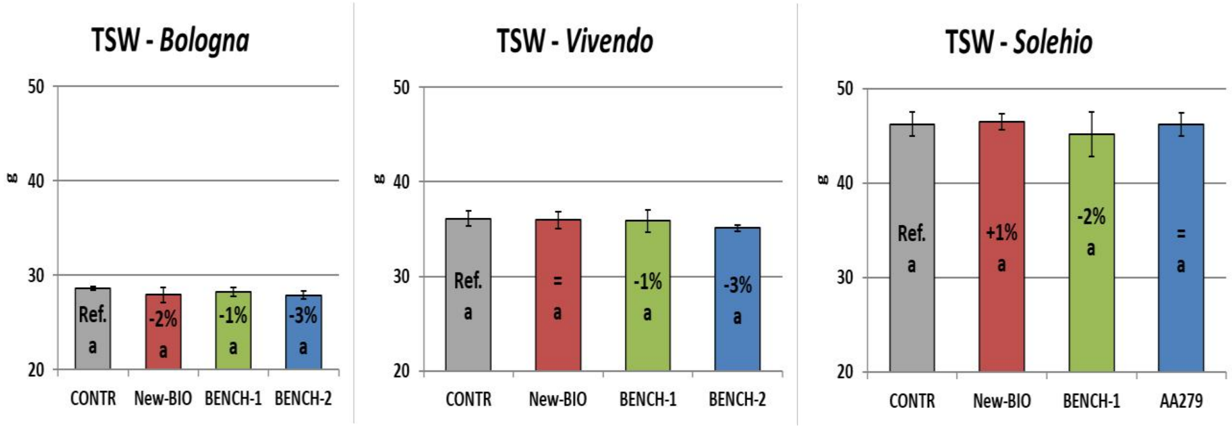


Fig 30: Thousand Seed Weight (TSW) of the three wheat varieties. Percentages inside histograms indicate the variation of each treatment vs. untreated controls. Letters indicate significant differences between treatments for each date (Student-Newman-Keuls test; *P*≤0.05).

The Thousand seed weight [TSW] of three varieties was recorded, and lower TSW was observed for Bologna compared to the other two var. Vivendo and Solehio. In Solehio, high TSW is recorded for Solehio variety in the new bio-stimulant treatment with +1% increase compared to uncontrolled treatment .

4.2.QUALITY PARAMETERS:

4.2.1 Grain Protein Content (GPC):

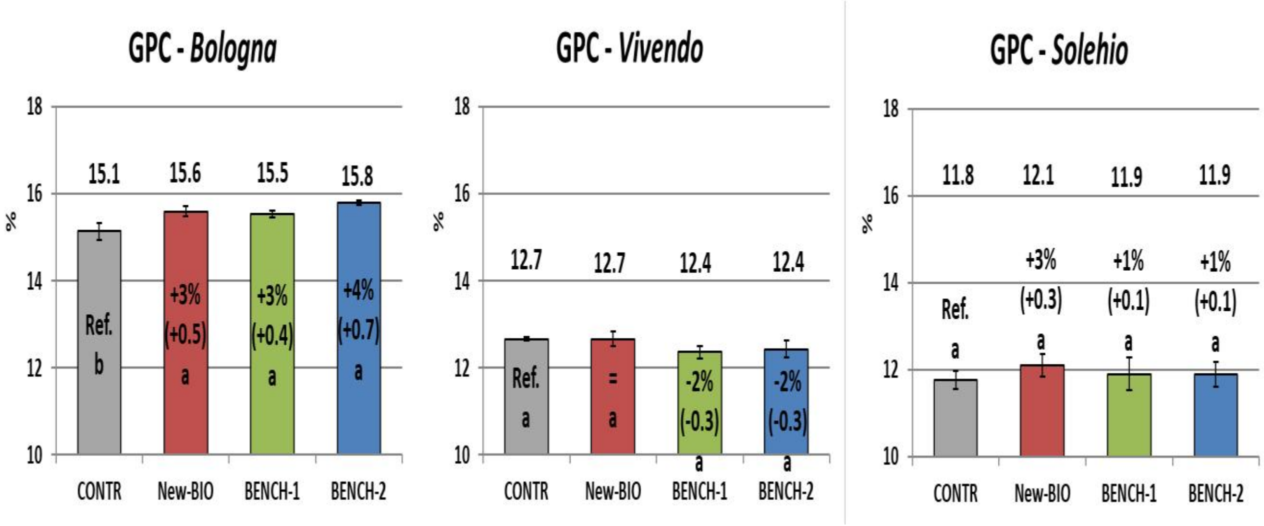


Fig 31: Grain Protein Content (GPC) of the three wheat varieties. Percentages inside histograms indicate the variation of each treatment vs. untreated control. Letters indicate significant differences between treatments within each variety (Student-Newman-Keuls test; P≤0.05).

Significant difference for Grain protein content in Bologna is registered, it being higher with 15.8 % in Bench 2 Treatment compared with other var.Vivendo and Solehio varieties. The lowest grain protein content is observed in Solehio variety in the untreated control with 11.8 %.

4.2.2.Gluten content:

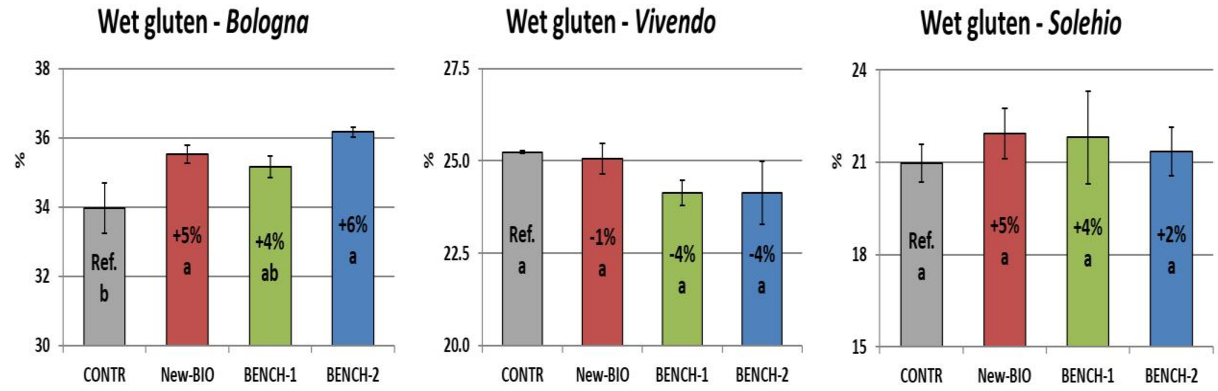


Fig 32: Wet gluten of the three wheat varieties. Percentages inside histograms indicates the variation of each treatment vs. untreated controls. Letters indicate significant differences between treatments within each variety (Student-Newman-Keuls test; P≤0.05).

The highest percentages of humid gluten content was registered again in Bologna variety with 36% in Bench 2 treatment compared with other var. Vivendo and Solehio. The lowest gluten content was observed in Solehio variety in the untreated control with 21 % decrease .

4.2.3.Zeleny index:

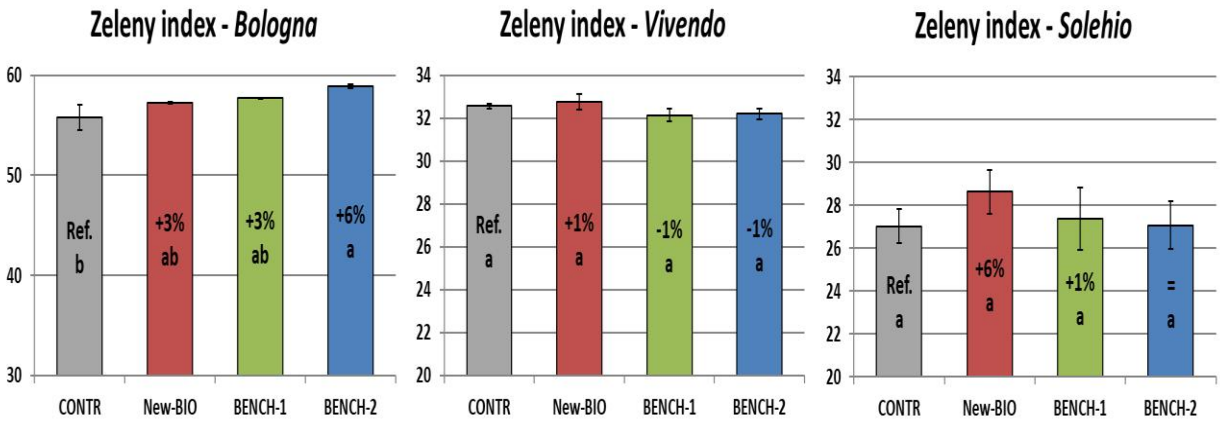


Fig 33: Zeleny index parameter of the three wheat varieties. Percentages inside histograms indicates the variation of each treatment vs. untreated controls. Letters indicate significant differences between treatments within each variety (Student-Newman-Keuls test; P≤0.05).

The Zeleny Index was compared for three varieties: Bologna variety is observed with high Zeleny index with >59 in Bench 2 treatment compared to the other Vivendo and Solehio varieties. The lowest Zeleny Index is observed in Solehio variety in in the untreated control with Bench 1.

4.2.4. Phenolic acid content in grains

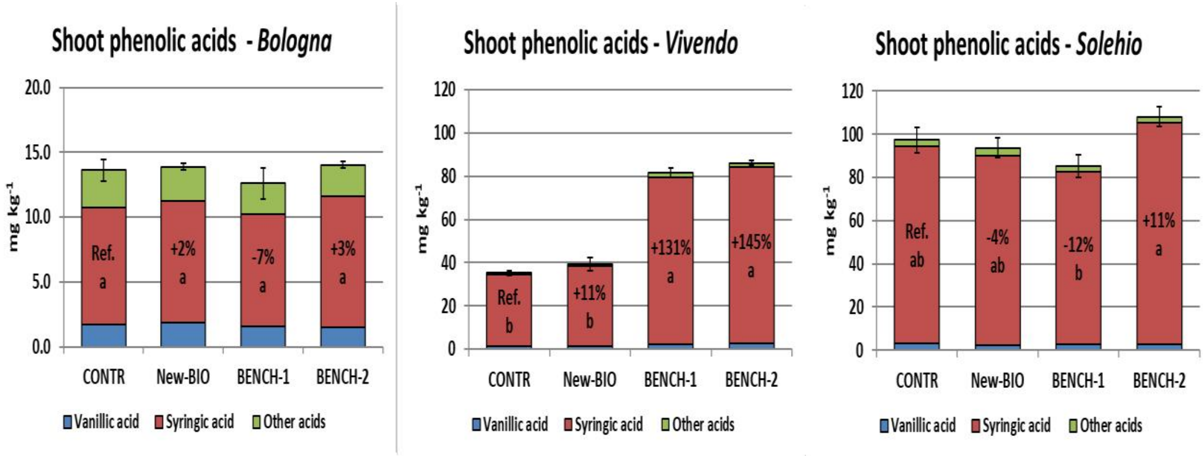


Figure 34: Phenolic acids content (vanillic acid, syringic acid, other acids: caffeic, *p*-coumaric and *t*-ferulic) in the grain of the three wheat varieties. Percentages inside histograms indicates the variation of each treatment vs. untreated controls. Letters indicate significant differences between treatments for each date (Student-Newman-Keuls test; P≤0.05).

Shoot Phenolic content was determined by HPLC of grain (flour)-methanol extracts of the individual treatments compared for these cultivated three varieties Bologna, Vivendo and Solehio. Shoot Phenolic content is higher in Solehio variety with a variability ranging from -4% [New Bio Stimulant] to +11%[Bench 2 treatment] compared to the untreated control, followed by increase in Vivendo variety ranging from +11% [New Bio-stimulant] to +145 % [Bench 2 treatment] compared to its control. Phenolic content includes Vanillic acid, which is higher in Bologna variety, particularly in New Bio-stimulant treatment with 2.5 mg kg^{-1}. In Syringic Acid, phenolic content is higher in Vivendo and Solehio varieties and the other phenols were very low in these 2 varieties. Other Acids [caffeic, *p*-coumaric acid sand *t*-ferulic acids] were higher in Bologna variety with almost similar values, in particular the other acids were higher in untreated controls and new bio-stimulants treatments of Bologna variety.

5. Discussion:

For many Nations, wheat is the backbone of their diets, thus we have chosen natural bio-stimulants as a way to boost wheat's metabolism, thereby increasing yields and quality. Successful results from using these materials have been reported by many growers worldwide. Growers of wheat are looking for cost-effective methods to boost productivity.

Research into the effects of exogenous bio-stimulants on the development of wheat was important because of their widespread usage in commercial agriculture to boost wheat output. All elements that affect plant metabolism as the plant is growing have an effect on yield, since yield is the total sum of all metabolic responses in the plant (Ibrahim, 1999). Wheat's yield potential is established by the number of spikelets, the number of viable florets, the number of grains per spikelet, and the size of the grains. During the tillering stage, wheat production is very sensitive to factors such as genotype, seeding rate, photoperiod, temperature, water, and nutrient status (Rajala and Peltonen-Sainio, 2000).

These results are part of a larger investigation of the effects of new bio-stimulants [sage and rice husk extracts] on three different types of wheat, including their susceptibility to environmental stress, their yield, and other aspects of their production. The aim of this trial was to verify whether a new bio stimulant applied at flowering of wheat can retard leaf senenescenza and improve yield and quality. The application of bio stimulants at this growth stage of wheat could replace late-season nitrogen fertilization, which is commonly applied to rise the protein content of wheat and dough strength, for a more sustainable agriculture.

Rice husk and sage extracts has abundant linoleic acids and bioactive compounds which are helpful in plants and humans metabolic reaction using them as a source of energy. Linoleic acid is a polyunsaturated fatty acid; like all the fatty acids, it can be esterified to form neutral, polar lipids such as phospholipids, triaglycerols and cholesterol esters. Linoleic acid acts as a structural component to maintain a certain level of membrane fluidity of the transdermal water barrier of epidermis. In addition, when released from membrane phospholipids, it can be enzymatically oxidized to a variety of derivatives involved in cell signalling. Linoleic acid is an essential nutrient that can be consumed by the human of all ages with certain limits to avoid chronic diseases.

The object of this study suggest that a foliar spraying containing bio-stimulants [derived from rice husk and sage extracts] applied to wheat during its flowering stage can have a beneficial influence on the plant's growth and development under adverse conditions and also serve as an antioxidant. During the grain-filling phase following bio-stimulant application, weekly detection of NDVI and SPAD measurements for canopy greenness and chlorophyll content highlight some benefits. Canopy greenness for the three varieties observed with similar values >0.6, with a minor difference in Bologna variety. Chlorophyll content was analysed for the Vivendo and Solehio varieties exclusively because of lodging issues with the Bologna variety. Particularly in the New bio-stimulant treatment of the Vivendo variety, high leaf chlorophyll values are maintained over an extended period of time.

Significant improvements in protein content occurred as a result of the crop's ability to maintain green the canopy for a longer period of time, photosynthesis during grain filling and nitrogen absorption. Here, the protein content is higher in the Bologna variety, with a high percentage [15.8%] in the Bench 2 treatment, followed by the slight increase in Solehio variety. Higher N levels in plant tissues are linked to these enhancements, which makes sense given that more protein means a lower SPAD value for grain yield. The new Bio stimulant significantly delayed leaf senescence that is commonly observed with the approach of ripening. With high protein content, wet gluten and the Zeleny index in Bologna variety are thought to be excellent parameters for super bread making quality.

Regardless of the type of bio stimulants applied, the results on yield were contrasting with Bologna and Vivendo (more protein-rich cultivars) experiencing a slight decrease, and Solehio experiencing a slight increase. It is likely that the unusual weather of 2021 had a major impact on the yield response, suggesting the trail extended into a second year.

It has been shown that a positive association exists between grain quality and testing weight, higher testing weights indicating higher grain quality and flour yield during milling. In general, the higher the test weight, the more endosperm rich starch and the less bran and hull are there in the sample. For livestock, farmers may find it more profitable to invest in grains with a higher testing weight due to their higher energy content, but not for huma nutrition.

A comparison of the three types of varieties for reproductive efficiencies using the Harvest Index shows that they are all similar, with Bologna variety showing the slightest decrease in the harvest index. The thousand-seed weight value is found to be greater for the Solehio variety.

Using an internal standard ratio and calibration curves derived from known standards, phenolic acids were measured by high performance liquid chromatography (HPLC). A control sample (MV-Emese) was tested multiple times during data collection from samples in the diversity screen to ensure data quality. Our findings are in line with those from earlier screenings of available cultivars, and they point up an intriguing observation: a rise in phenolic content in the flour. The phenolic acids ferulic acid, vanillic acid, and caffeic acid are all typically present in whole grains. In our research, we found that the Solehio variety is observed for its high phenolic content and especially with high syringic acid content but low vanillic acid and other acids. Aleurone cells and other exterior layers of the grain that form the bran following milling contain a high concentration of phenolic acids, as is widely known. Antimicrobial, antioxidant, and anti-inflammatory effects are only a few of the many biological actions attributed to phenolic compounds.

Canopy greenness, yield, and quality metrics are all affected by a number of variables. In addition to factors such as temperature and water, soil fertility, variety, and sunlight, the quantity of tillers can be influenced by these environmental factors. In addition to these parameters, adequate fertilisation and nutrient absorption can boost tillers with high protein content and tillers with high grain filling capacity, resulting in a noticeable improvement in quality and production. With high photosynthetic efficiency or dilution in protein content or poor nitrogen absorption can also raise yield but contain lower protein content. The protein content of grains can be increased if the nitrogen levels are optimal, and lower yields if they does not meet favourable conditions.

6.Conclusions:

Attempts have been made to analyse the parameters for canopy greenness, yield, and quality, as well as to assess the changes that have occurred in the physical-chemical properties of wheat crop. New bio-stimulants treatment has performed high greenness canopy in all three kinds, and increased chlorophyll content in the Vivendo variety compared to benchmark 1 and benchmark 2 treatments. Contrastingly, yields were decreased, although in the low-protein content variety Solehio there was an appreciable increase (+8-10%). Additionally delivered a quality performance but still more research has to been carry in the next future.

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