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Modern Phenotyping: A Paradigm of Present Need in Crop Improvement

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Modern phenotyping is intended to develop and establish the understanding of quantitative traits. Development of crops enriched with improved stress resilience and high yield potential, made it necessary to look into plant biological process that are responsible to crop improvement. Plant breeding aims to accelerate the genetic gain by utilizing modern high throughput phenotyping approaches which established the association between genotype and phenotype over the time and offers also elucidation of complex plant characters/phenotypes. Therefore, modern phenotyping or high throughput is forefront of future crop improvement. The major advantage of this innovative technique is monitoring of plants through nondestructive manner using effective imagining methodologies to gather the data for studies of complex characters in quantitative manner which

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are directly or indirectly related to plant yield, growth and to combat against biotic and abiotic stress. With a view to above facts this paper explains modern phenotyping approaches for crop improvement.

Keywords: Phenotyping; precision agriculture; NDVI; DSS; UAVs.

1. INTRODUCTION

Agriculture is the ultimate source of food production to feed the burgeoning population but shrinking agricultural land, climate change and ever augmenting world population are some of the serious menaces that needs to be addressed. For this, horizon of research needs to be expanded and improvement in yield potential and adaptation to stressful environments to sustain the food security has to be accelerated [53]. Despite of this, conventional agriculture uses excess amount of chemicals like fertilizers and pesticides to achieve the expected yield resulting into degradation of environment and evolution of pathogen strains resistant to pesticides. In order to meet these wide ranging global concerns, advanced approaches or tools are needed to improve the quality and productivity of crops. This entire scenario has drawn the attention for identification and analysis of quantitative phenotypes and to describe the genetic basis of crucial traits to accelerate the selection of plant that can thrive well in resource limited environment [30]. Molecular breeding approaches are concentrates on genotypic selection but still, these molecular techniques requires phenotypic data to support the genetic information [43] because, (1) phenotypes helps in selection and edifies genomic selection based on prediction model (2) In marker based recurrent selection, markers for following selection in generations, can be identified in a single phenotyping cycle [63] and (3) During transgenic studies, phenotyping is imperative for identification of propitious events [34].

Current phenotyping approaches are times consuming and is rely on visual scoring, which are prone to biasness between different experimental repeats. Advent in the molecular field specially in sequencing technologies provided almost limitless access to high density genetic markers which are really helpful to establish association between genotype and phenotype for quantitative traits at genomic level. But error free recording of major important traits and crop monitoring through conventional phenotyping techniques still remains bottleneck in plant breeding[56, 59] and needs to be synchronized with the fast paced evolution of molecular field to achieve the more genetic gain. Trade off between accuracy and speed is a major restraint in conventional phenotyping of complex traits. This bottleneck can be overcome with the help of modern phenotyping tools which are high through put in nature and gives enhancedspatial and temporal resolution [32,92].

During recent years, these approaches have drawn major attention and established of new protocols/techniques to observe and record different plant traits [16]. Plant phenotype is the product of interaction of genotypes with its local vital and dynamic environment in both the sphere (spatial and temporal). Plant phenotyping can be explained as the evaluation of complex plant traits such as growth, development, tolerance, resistance, architecture, physiology, ecology, yield and the basic measurement of individual quantitative parameters that form the basis for complex trait assessment [52]. Manual phenotyping of traits on single plant is more precise than modern phenotyping, if done accurately, but much slower. Modern phenotyping or high-throughput phenotyping approaches use different types of spectral and thermal sensors and fluorescence (for proximal or ground based phenotyping) and imagers (for UAVs), which are mounted in or on remote sensing devices. These sensors traps radiation emitted by the canopy [4]. Application of these modern devices for field phenotyping is discussed comprehensively in the various literatures [24-25,28,68,89]. Phenotyping through imaging offer opportunities to uncover the plant attributes like photosynthetic rate, growth rates and root physiology in non invasive manner via scanning temperature profiles [29].

Canopy temperature (CT) and Normalized Difference Vegetation Index (NDVI) are two traits which are commonly screened through modern phenotyping tools. Canopy Temperature strongly associated with plant performance under stress condition, since it has direct correlation with status of water in plants and stomatal conductance [2,9,11] while NDVI can be used to appraise relative biomass of plant [5], nitrogen deficiency and crop senescence rate

(5,11,66,74,76]. In plant breeding thousands of germplasm lines are evaluated in small plots for various traits, but in precision agriculture, crop is evaluated for different types of stress and weeds at an early stage in large fields. Therefore, objective and approaches of phenotyping unlike for plant breeding and precision agriculture and have different requirements [16]. In both scenarios, modern field-phenotyping tools/approaches are required.

2. MODERN PHENOTYPING TECHNIQUES

Plant breeding approaches are directed to create new cultivars which can thrive well in target environment. Competency of selection is evaluated in terms of increase in frequency of favorable alleles, for which, proper screening and précised selection of genotypes is pre-requisite. Similarly, altering the genotypic structure by changing the gene frequency is based on precise phenotyping and selection. Plenty of germplasm lines are evaluated for various agronomic traits in small plots ranging 1 to 10 meter in length. Yield trials are replicated and conducted in multi-environment to appraise genotype × environment, resource and crop management interaction [41]. Evaluating the large number of plots for different traits in different environments in time bound and resource limited environment and quality of the data is the major challenge in phenotyping in plant breeding. High-throughput phenotyping is a speedy and non-destructive strategy for screening of plants [92].Modern Phenotyping is capable of characterizing plant traits in considerable size of populations in both time and space which results into effective surveillance of genetic responses to environmental conditions [90]. Advent in modern phenotyping technologies and data processing has opened up new avenues for crop improvement in field as well as controlled environments [3, 45,50, 61,87]. Thus, high-throughput phenotyping delivers the scope for greater selection intensity, upgraded selection accuracy and refined the decision support system [4]. HTPP holds the great promise to revolutionize the farming especially precision agriculture by detection of pathogen and pest [57]. The commonly used modern phenotyping approaches are:

2.1 Satellite Imaging

Satellite imaging is acquirable with multispectral spatial resolution which ranges in between 1.24 meter to 260 meter. Data from medium resolution satellite freely accessible but high-resolution satellite data can be accessed commercially. Breeding programs generally carried out to evaluate plenty of genotypes in approximately one meter sized plots, especially during the early selection cycle. Such small size plots needs satellite images with higher resolution and currently available advanced satellite sensors fails to deliver such high resolution. Thus, for plant breeding trials, satellite imaging can be used only to evaluate moderate to large sized trial (yield trials). Yield trials are multi-location trials which are replicated at various locations to assess the G × E interactions.

Multi location trials are often planted at different locations throughout country which makes them difficult to evaluate through proximal or drone-based approaches. Therefore, satellite imaging could be viable for these multi location trials considering the reduction in cost of imaging. Appropriate plot size for breeding at particular resolution was evaluated in wheat germplasm with the help of Digital Globe World View-2 satellite which has multispectral resolution (spatial) of 1.84 m ground sample distance (GSD) and it was concluded plot size which could be analyzed should not be smaller than 2 × 0.8 m while 8.4 × 2.4 m plot size is appropriate at that resolution [89]. Large areas can be covered with satellite imagery at a time as compare to proximal phenotyping which has the chances of lose of precision due to coverage of small area instantaneously. The phenotyping through satellite imaging is hampered by factors like weather conditions, frequency, resolution and imaging cost. In plant breeding, low altitude airborne imaging which is also a unmanned aerial sensing, overcome the bottleneck of resolution at plot level while providing the opportunity of surveying number of plots concurrently at elevated temporal resolution [3,14].

2.2 Unmanned Aerial Systems (UAS)

In field experiments with number of plots or large-breeding nurseries, Unmanned or uncrewed aerial systems (UAS) serve as a possible substitute to ground-based phenotyping platforms in sensing technology and data analysis [71] and available at low cost as compare to satellite imaging and has great potential to play an important role in plant breeding in genomics era for precise quantitative phenotyping of complex traits. Obviously, the

UAS approach has potential to increase throughput phenotyping but low-cost consumer-grade sensors and platform should be developed and acquired precision in comparison to other approaches is also an affair of study [85]. UAVs falls broadly into four categories viz; (a) Parachutes (b) Blimps, (c)Rotocopters and (d) Fixed wing systems [82]. Although, application of UAVs in agriculture, especially in plant breeding, for the purpose of phenotyping is relatively new and slowly increasing [14,26,51,96] but still, worth of spectral indices gleaned by airborne system has been documented by many scientist to evaluate the environmentally determined traits in different crops [13,10,27,38,84,87,97,99].

UAVs based selection of elite breeding lines and clones offers an opportunity for enhancement of germplasm and genetic gain if low cost UAV platforms and proper protocols for imaging and analysis can be developed. Tattariset al. [89] compared high through put approaches viz; low flying UAVs, proximal sensing and satellite imaging while considering CT and NDVI to discern the most feasible approach for phenotyping. The UAV-based platform showed higher plot-level resolution at an altitude of 30–100 m while measuring several hundred plots at a time and hence, supported the advantage of UAV-based phenotyping to pull off the precision and efficiency. UAS offers few advantagesdepending upon the aircraft, sensor and platforms being used and objectives [72].

Unmanned Aerial Systems are efficient enough to work and capture images in fluctuating weather conditions in contrast to satellite imaging. The chief limitation of UAVs is their higher cost although, low cost UAS is also available but weight and dimension of anchored sensors is not up to the mark. Low cost UAVs show limitations in stability, accuracy and reaching at certain altitude [78]. On the other hand, factors like lens distortion, overlapping of acquired images during airborne time and camera positions during image acquisitions can

Table 1. UAVs used to study different traits in various crops

S.N.	Type of UAVs	Trait/Crop Studied	Scientist
1.	Octocopter at an altitude between 30 and 100 m	Experiments in spring wheat for yield and biomass	Tattaris et al. [89]
2.	Octocopter at 15 m flight height.	Cotton trials	Xu et al. [94]
3.	Hexacopter at 25 m altitude	Drought tolerance in black poplar	Ludovisi et al. [54]
4.	Rotocopter at an altitude between 20 and 30 m	Vegetation indices was estimated in wheat trials	Khan et al. [47]
5.	Hexacopter at an altitude of 50 and 75m	Plants height in wheat	Madec et al. [55]
6.	Octocopterat an altitude of 50mto digitally	Plant population in maize	Gnadingerand Schmidhalter [37]
7.	Hexacopter from 50 m altitude	Grain yield in maize	Geipel et al. [35]
8.	Octocopter at 50 m altitude	Biomass and Plant height in barley	Bendig et al. [8]
9.	Pheno-copter	Studied ground cover, canopy temperature and crop lodging in sorghum, sugarcane and wheat respectively	Chapman et al. [14]
10.	Two quadcopters	Crop lodging in wheat	Singh et al. [85]
11.	Fixed wing gliders	Nitrogen stress variation in bread-wheat germplasm	Zaman-Allah et al. [96]
12.	Fixed wing gliders	Height of sugarcane plants	De Souza et al.[22]
13.	Fixed wing gliders	Phenotyping of small sized wheat breeding plots	Lelonget al. [51]

cause flaws up to certain limits in orthomosaics (Interactive image map) generated by UAVs. In spite of this, more technical aspects such as high-speed ultra-low situation, efficient data downloading and the software for its automatic processing to secure real-time application, payload to be carried, needs to be considered before using UAVs [98].

2.3 Proximal Phenotyping

Fieldbased modern plant phenotyping offers opportunity for noninvasive quantification of plant structure and function and assessment of their interactions with environments. Phenotyping of plants with the help of ground-based platforms is known as proximal phenotyping. Proximal phenotyping also uses sensors which can be handled or can be installed on moving platforms (vehicles) or stationary platforms such as towers and cable suspensions [24]. NDVI reveals the photosynthetic efficiency of the plant because it is related to chlorophyll content and can be determined in the electromagnetic spectrum (near infrared and visible regions). Canopy Temperature can be indirectly used to measure the transpiration rate of a plant and ascertained from emitted infra- red radiation [9,70]. Traits like NDVI and CT can be phenotyped effectively using ground based proximal sensing approach while handheld sensors can be used to evaluate the traits like chlorophyll fluorescence, nitrogen content, leaf area and height of plants. On the other hand, mobile platforms are being developed and tested to determine the biomass, population, height, early vigor and maturity of a plant. Mobile platforms motion and navigation can be done manually or can be motorized supported.

In plant breeding, plants are evaluated for multiple traits based on needs such as morphology, tolerance to stresses and phenology. This approach provides the opportunity to evaluate the plants for multiple traits at a time. Handheld sensors are useful to evaluate small number and size of plots. But, in case of large number of plots, handheld sensors are less efficient because of temporal errors. This bottleneck can be removed with the help of

Table 2. Proximity sensors used for study of various important traits

S.N.	Type of Proximity sensors/instruments	Trait/Crop Studied	Scientist
1.	Infrared thermometer (Handheld)	Drought tolerance in maize	Keener and Kircher [46]
2.	Spectro-radiometers (Handheld)	Number of morpho-physiological traits, disease like Yellow rust and nutrient use efficiency such as nitrogen use efficiency in wheat	Kocet al.[49], Garrigaet al. [33], Huang et al. [42], Odilbekov et al. [64]
3.	Chlorophyll meters and chlorophyll fluorescence meter (Handheld)	Plant health, photosynthesis, status of plant nitrogen and yield and yield attributes in several crops	Keener and Kircher [46], Odilbekovet al. [64], Debaekeet al. [23],Yang et al. [95] and Chawade et al. [17]
4.	Phenocart (Mobile platform)	Correlation of grain yield with canopy temperature and plant health.	Crain et al. [21]
5.	A proximal sensing cart (Mobile platform)	Breeding nurseries of wheat, barley, camelina and cotton.	White and Conley [93]
6.	Modified version of PSC (Mobile platform)	Drought tolerance in cotton germplasm	Thompson et al. [90]
7.	Phenomobile (Mobile platform)	Canopy height and temperature, number of spikes in wheat and plant stress	Deery et al. [14]
8.	PhenoTrac 4 (Mobile platform)	Evaluation of wheat and barley breeding nurseries for uptake of nitrogen, grain yield and dry weight	Barmeier and Schmidhalter [6-7], Rischbeck et al. [77]
9.	GPhenoVision (Mobile platform)	Canopy growth and development in a cotton breeding trial	Kirchgessner et al. [48]

mobile platform which are capable of evaluating number of traits and rows at a time and results into saving costs and time [16]. Non-automatic platforms have additional advantages over motorized platforms because (i) they are economical and (ii) comparatively simple to develop. Motorized platforms are heavy weighted, carry more sensors and needs onboard energy source to move. However, motorized platforms are requires technical skill and are expensive [16]. Number of studies has been taken to evaluate the different traits in various crops using this approach, account of which is summarized in brief as:

3. MODERN PHENOTYPING TOOLS AND GENETIC GAIN

Although much success has been witnessed in last few decades in terms of yield and tolerance/resistance against biotic and abiotic stresses but, genetic gain in yield of major crops has been stabilized or stagnated in spite of modern scientific advances [1, 81]. This stagnation draws attention towards urgency to increase the efficiency of breeding approaches. Small breeding populations have very low frequency of favorable alleles and imprecise phenotyping in such small population will belittle the genetic gain. Less efficient phenotyping in plant breeding is considered as one of the key constraints to achieve the genetic gain [3, 36, 88). Breeding programme can be accelerated to increase genetic gain in a number of ways [58] like (i) Higher selection intensity (ii) Précised selection (iii) Enough variation in genetic component (iv) Accelerated breeding cycles and (v) improved decision support systems. Reliable high-throughput phenotyping is the key requirements for all approaches mentioned above in either direct or indirect manner. Phenotyping is not just a recording of data or measurement of traits; proper phenotyping requires appropriate and spatial variability handling and trial management in a resource efficient manner and the development and management of more comprehensive data. Various study supported increase in genetic gain upon integration of genotypic data with high through-put field phenotypic data. Grain yield in wheat was predicted with the help of genomic prediction models by integrating HTPP data (obtained by UAVs) and genotypic data and found accuracy was lying between 56 to 70%[80]. In another study, HTPP data on NDVI and canopy temperature, was collected with Phenocart (UAVs) and proximal phenotyping and was integrated with genotypic data to evaluate the genomic prediction model and achieved accuracy was 7% [20].

4. PHENOTYPING FOR PRECISION AGRICULTURE

Concept of precision agriculture was established during late 1970s when it became possible to ascertain correct position of a point or location in terms of latitude, longitude and altitude at given time due to development of the global positioning system [86]. It is an integrated approach of various components in which, technology and high-resolution data is key integral part to monitor and manage the field so that output can be maximized and inputs can be minimized [12]. Precision agriculture works with optimized factors. Possible factors that can be optimized to achieve higher yield are controlled chemical spray (Fertilizers/pesticides/herbicides), control of disease, weeds and irrigation and optimum plant density. Optimization of these factors is possible through the sowing and fertilizer spraying equipment equipped with advanced sensors [16]. Phenotyping in precision agriculture is focused on improving management practices while in breeding; it aims in the selection of relevant genotypes [56]. Thus, needs for phenotyping is different in precision agriculture than plant breeding thus called for different strategy and solutions [15]. Management of pest, fertilization and irrigation is the key factor which that can be dealt with the help of high through-put field phenotyping. Satellite imaging data is integrated with weather data to develop the prediction model in precision agriculture.

Physical loss due to different types of stresses (biotic and abiotic), fluctuating rate of fertilization and irrigation to the crop can be determined with the help of satellite imaging. Factors like revisit frequency of satellite, available spectral bands and imaging resolution plays a key role in use of satellite imaging in precision agriculture. Higher revisiting frequency is utmost important in precision agriculture as relevant farm decisions are time sensitive [16]. Since, sensors and carriers are trait specific hence; it is peculiar to choose the right sensor and carrier for the individual trait. To customize the action, retrieved image is supposed to be linked with geographical information. Early detection and rapid analysis is pre-requisite to prevent losses[16]. In precision agriculture, to predict the urgency of the action, Decision support systems (DSS) with phenotype data should be linked to weather data. High through-put phenotyping varies according to crop, trait and availability of resources. HTPP can also serve to broaden the genetic variation by evaluating the germplasm or breeding material for such traits that are invisible to naked eye and then they could be retained in the breeding programs [80]. Utility of modern phenotying tools in precision agriculture can be utilized for:

4.1 Optimizing Fertilization

Scattered distribution of nutrients is common phenomena of soil. Therefore, an application of fertilizers with uniform rate is inefficient, uneconomical and liable to have adverse effect on the soil and environment along with quality and quantity of produce [83]. In case of optimized fertilization variable rate of nutrient are applied based on the results of analysis of soil samples to reduce the loss of nutrients [75]. HTPP is more crop-centric approach in which, plant phenotyping is done using optical sensors to ascertain the status of nutrients of field and fertilizers is distributed in accordance to the derived information [96]. Ability to separate different types of nutrient deficiencies and certain disease or combinations of both at phenotypic level remains a challenge to optimize the fertilization and it can be settled by using amalgamation of different vegetation indices and wavelengths to make this distinction [57].

4.2 Detecting diseases and pests

At present, fields are manually surveyed for disease which is laborious and time consuming. Modern phenotyping tools can push the crop protection to a new level by automated early diagnosis of diseases in the field which would serve as warning system thereby customize the action needed to reducing crop losses and lowering down the need for pesticide. Furthermore, disease monitoring stands as one of the key principles in integrated pest management (IPM) and its application in the field can be simplified and amplified [16]. Effective identification of disease through high-throughput techniques remains a challenge. Recent developments in processing of image such as deep and machine learning of big data have brought the effectiveness in identification of crop and pest simultaneously [31,60].

4.3 Detecting Weeds

Weeds can cause greater yield loss compared to pests and pathogens [65]. At present, spraying of herbicides is commonly used method to combat yield loss. However, due course of time, resistance against herbicides have been fetched by several species of weeds because of excess use of them [40]. It is inefficient also to spray in whole field with herbicides against a small affected patch of the field. Scientists have achieved the higher accuracy in weed detection using modern phenotyping approaches. For example: Pena et al. [69] scored up to 91% efficiency in sunflower fields with the help of a UAV. Blue river technologies (Sunnyvale, CA, USA) designed a customized tractor with mounted sprayers which identify the affected area from weed and selectively spray herbicide in real time [15].

4.4 Decision Support Systems (DSS)

The success of precision agriculture is directly related to rapid and précised analysis of obtained phenotypic data. Acquired Phenotypic data of complex trait has to be processed and analyzed rapidly in a sturdy manner to derive useful and needed information. It requires integrated approach involving knowledge of biometrical genetics, statistics, geneto-phenotype with genomic selection etc. [18,88]. It is known that final achievement or gain not depends on improved highquality sensors or improved platforms but it is a data-driven approach and subjected to data quality and advancement in data processing [18, 91]. DSS is capable of combining the phenotypic data with weather data or market data and possible information regarding disease to optimize the yield and or farmer’s profit. Even though, there are tremendous opportunities to combine different types of data to optimize the yield but still, the use of DSS which rest on phenotypic data is very limited. Embracing and utilization of DSS by the farmers is remains very low and is another aspect of research [79]. Collection, precise classification and tailoring this data into useful and reliable information and or decision are also a matter of extensive research. Key factors of negligence or poor adoption of DSS by the farmers are less stable performance, difficulties in use, cost, habit, relevance and trust in DSS. Other factors are age of the farmer, land holdings and education level of farmer.

Modern phenotyping tools opens up new avenues in the parameterization and configuration of models with the help of genetic inputs [67]. Even though, many DSShave been developed, very few DSS focuses on (automated) phenotyping. Focus should be placed on key factors while developing the DSS. In Tanzania, an open access mobile application has been developed and tried out to detect the symptoms appearing on foliage in cassava using deep machine learning [45,62,73] developed a framework (which works on phenotyping) to determine the stress caused due to iron deficiency chlorosis in soybean. In this line, Hallau et al. [39] also developed an application that takes the picture and was able to detect and distinguish number of foliar diseases including bacterial blight of sugar beet, leaf spot caused by *Cercospora* and beet rust.

5. AFFORDABLE APPROACHES

Espousal of modern phenotyping approaches or tools is supposed to call for huge commencing investment specifically for testing networks and establishing its utility over the large geographical areas. Key challenge to accelerate the development and adoption of modern phenotyping is to develop the tools/techniques which are capable of delivering the reliable performance across the large geographical area in affordable cost especially in low or middle-income countries where labors are available in relatively lower emoluments as compared to higher income countries. Generally field-deployable vehicle on which robust sensors are mounted or proximal phenotyping is supposed to be imperative for field-based phenotyping than other approaches available for high through-put phenotyping [68].

6. CONCLUSION

Crop improvement is very important strategy in order to increase the production and useful for food security globally. The modern and innovative techniques like UAV (unmanned aerial vehicle) will improve the field management thus increase the productivity. Development of decision support system through robust sensors will improve the efficiency of phenotyping. Exploiting a single technology always a limiting in improving the crop therefore integrated approaches will help farmers in field operations. This review explains about various wavelengths imaging technology in phenotyping. The important pre-requisite in collection of phenotypic data is sensors being used for imaging in plant phenotyping, physical properties, acquaintance with trait and depth of knowledge, software technology and analysis of obtained image. Every modern phenotyping tools used for specific traits like fluorescence imaging and thermal imaging mainly used for detection of foliar disease and plant water status respectively, 2D visible imaging for estimating shoot biomass, growth patterns and 3D imaging for biomass estimation. The challenge in modern phenotyping is to develop the concern software tools to further analyze the data and to obtain the physiologically interpretable data. With the improvement in the current imaging and refinement in new technologies will helpful in dissecting plant phenotype. One of the key examples of this modern technique is precision agriculture where satellite imaging is supporting to strengthen the decision.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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