Collection of quantitative images of leaves in the field and greenhouse

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This note describes the photographic apparatus and procedures we have used to collect quantitative images of leaves from les mutant plants in Missouri.

Apparatus. The apparatus consists of an imaging platform, a digital single-lens reflex (SLR) camera, and a means to evenly illuminate the platform.

Platform. The platform joins a photographic plane and a support for the camera. The plane is a sheet of 3/8 in plywood, 14 3/16 x 10 3/16 in, screwed into a V-shaped aluminum bracket. The bracket's base extends beyond the platform and admits a 1/2 x 48 in steel rod; a set screw holds the rod in place. 3/16 in thick foam board (Borden Elmer's), cut to match the plywood, is covered with blue cloth; the cloth is taped to the back of the foam board with duct tape. The covered foam board is mounted to the plywood using four small pieces of industrial-strength velcro. (We change the entire foam board as the cloth gets dirty.) On the side of the cloth facing the camera, a GretagMacBeth mini-color checker (now a Munsell color checker available from X-Rite, # M50111) is mounted on a small piece of velcro so that it is approximately 1.25 in from the edge of the platform next to the bottom of the V, and centered left to right. The color checker is mounted so that its greyscale is toward the rod. The cloth is blue, matching the blue square of the color checker as closely as possible (RGB = (56,61,150)).

The camera is suspended from a Manfrotto ball-jointed, quick release tripod mount (# 486RC2). The joint comes with the corresponding quick release plate that threads into the base of the camera. The threaded end of the mount is screwed onto a 1/2 x 6 in aluminum rod. This short rod is clamped to the steel rod using a perpendicular rod clamp. 1/4-20 x 5/16 in cup-point alloy steel hex socket set screws are used in the clamp and at the base of the V to tighten joints. The rods and ball joint are arranged so that the platform just fills the camera's field of view and so that the plane of the camera lens is parallel to that of the platform. Once set, the suspension need be checked only at the beginning of a photographic session. The platform and key components are shown in Figure 1.

Camera. We have used Nikon D70S (2006) and D80 (2007) cameras, both with a fixed lens. We strongly prefer the D80: the autofocusing algorithm is much faster and more robust; data collection and writing to the memory card are faster; the noise correction algorithm is substantially better; and the camera's resolution is much higher (10 MP). A 60mm f/2.8D AF micro-Nikkor lens (# 51214) is used with a UV filter (lately, a ProMaster digital). This

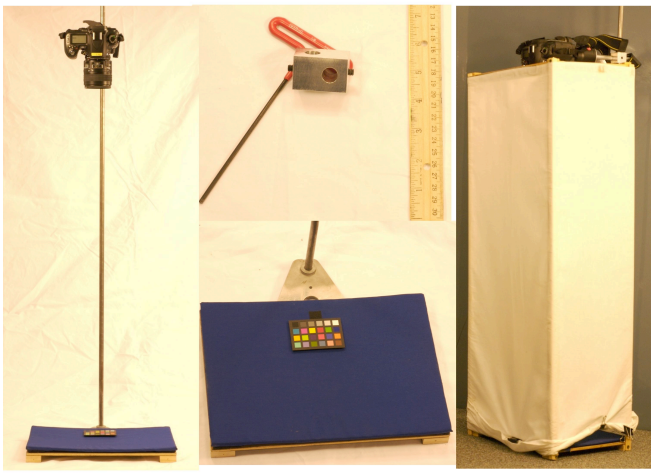

Figure 1. The imaging platform. The left panel shows the unshaded platform used in the greenhouse and for time-course photographs in the field. The middle panel shows the perpendicular clamp and its wrench (upper) and the platform bottom (lower). The right panel shows the shaded platform for collection of end-point photographs of detached leaves in the field (the "box kite"). (For full color, see p. 32.)

lens combines good field of view with depth of field, helping to project the waviness of the margins of the mature leaf into the photographic plane. Since we minimize the distance of the lens to the leaf, mature leaves must be shot in three sections, to be joined together digitally in the laboratory.

If the leaf is centered in the camera's field of view with the color checker close to it, we have found we can rely on the D80's autofocusing, but not on that of the D70S. We routinely check the focus prior to shooting each leaf, however. Data upload to the computer is via the camera's USB port.

All of our images are collected in RAW format at the highest possible resolution, and we under-expose the images by 2 - 3 “bars” on the camera's internal light meter. In the 2007 field season, the remaining parameters were ISO 200-400 to minimize noise; f8-11 for good depth of focus; and shutter speeds between 1/20-1/160. We adjusted the white balance periodically throughout the day using an ExpoDisk digital white balance filter, shooting the platform shade as a standard.

Illumination. All photographs to date have been taken using reflected light, except for a few experimental transmitted light ones. It is imperative that the leaf and platform be evenly illuminated, with no dark or light areas. In the field, we use ambient light exclusively: the waxiness of the leaves produces bright spots unless a flash is directed at the underside of a reflective umbrella, and in that case the quality of the illumination was not improved. In the 2006 field season, photography was confined to the morning (ca. 0800 - 1200) and late afternoon (1600 - 1900) to avoid excessively warm or cool light. In the 2007 season, we were able to shoot throughout the day provided we adjusted the white balance while the light color was rapidly changing.

In the field, even illumination is provided by carefully shading the platform. For time-course photographs, this has been done by using one to two photographic umbrella(s) to shade both the camera and the platform (2006). On bright, clear days this method

works well, but it fails badly on overcast days: the light is reflected by the clouds so much so that it falls on the platform from all directions. In that case, our only recourse has been to surround the platform with people to block the light. We are now considering alternative shading devices for the 2008 field season (essentially, a lightweight beach umbrella with sides). For end-point images, we circumvented this problem by surrounding the platform with partially opaque drapery liner cloth (Roc-Lon rain no-stain # 184-9801), stapled to a wooden rectangular frame attached to the platform and open on its short sides to permit one to slide the leaves onto the platform (the "box kite"). The cloth hangs approximately 6 in below the platform, and the top of the kite, camera, and photographer's head are covered with a large piece of the same material. The wooden frame does not cast any shadows on the platform, even in very strong sunlight.

Since the greenhouse is designed to reflect light from every possible surface and the color of the light from the grow-lights is too orange (even with a yellow filter), we built a 68 x 60 x 115 in tent of drapery liner (more opaque than that used in the field, Roc-Lon special shade white # 796-2517). Sections of fabric were glued together with heat-sensitive fabric glue to avoid introduction of light through needle holes. The drape was tacked to the frame on three sides, with the fourth side suspended from a rod by shower curtain rings so that it opens easily to permit people and plants to move in and out of the tent. The lightweight tent is suspended from the greenhouse's structure (we use an electrical conduit) by a series of pulleys and is lowered away from the lights when not in use. Light is supplied by 4 to 6 150-200 W fluorescent bulbs that fit an incandescent socket (e.g., n:vision) in aluminum or white plastic clamp reflectors mounted inside the tent, either on light stands or a frame attached to a small sawhorse. The lights are aimed so that they cross-illuminate the leaf to prevent shadows.

Leaf Preparation. Several minutes before photography, leaves are cleaned by spraying them with water from a hand-held misting sprayer and wiping them dry with a soft cloth. The leaves are wiped from the culm to the tip, one side at a time. A heavy dew usually suffices to clean the leaves. Only the top surface is sprayed; the bottom surface is wiped with the damp cloth. Leaves are then left to air-dry before imaging.

To provide an external reference for joining leaf sections, in the 2007 field season we taped one to two fiducial marks to the underside of the leaf. These project 0.5-1 in perpendicular to the length of the leaf, on the side opposite the color checker, and are placed so that they evenly divide the leaf. The marks should be red: we used 2 in lengths of 22g insulated wire attached with 3M gloss finish multitask tape (# 34-8507-5365-7). With care, the same tape can be reused several times before replacing it.

While the box kite shades the platform extremely well, it prevents all members of the photographic team from seeing the leaf, making photography very slow and clumsy. For end-point photographs in 2007, we therefore cut the clean leaf from the culm using a small pair of bandage scissors less than a minute before it was to be photographed.

Photography. Photographs are collected by three to four person teams. In the field, the platform is either carried to the plant to image the leaves in situ (both time-course and end-point photographs, 2006), or cut and brought to a photography station set up between the rows (end-point, 2007). For in situ photography, one person holds the platform, one the leaf, one the umbrella, and the fourth keeps other plants out of the way and records the data. Everyone sees the leaf on the platform and the field of view of the camera, and checks leaf alignment, lighting, and obstructions. The shutter is triggered by an infrared remote control. For ex situ photography, two folding lawn chairs are placed a platform's length apart; one person holds the leaf on the platform, resting the platform on his knees, and records the image number for each successful shot while the other photographs it. The photographer must direct the leaf-holder in aligning the leaf, since only he can see it. The camera's shutter button is used. The third person cleans and collects leaves and records data on the plant and leaf.

In the greenhouse, plants are placed on plant caddies and rolled to the tent for in situ photography. Short plants are brought completely into the tent, while tall plants are tipped in through the curtain. The platform is placed so that its angle is parallel to that of the plant. For short plants, it is simply held as in the field; for tall plants, it rests on a sawhorse at a constant position, marked by duct tape on the 1/2 in steel rod and on the sawhorse. The photographer stands on a box, if needed, and at a marked spot behind the sawhorse, so that the angle of the platform relative to the lights is constant.

For all images, the leaf is placed so that its long axis is parallel to the long axis of the platform; adjacent to the color checker; and with its tip at the leaf-holder's right hand. The leaf is gently flexed and unrolled so that it is as flat as possible over as much of its length as possible; the presence of fingers in the image is minimized. A leaf is photographed in as many sections as needed to cover its entire length (1-4), but always starting at the tip end and finishing at the culm. We make sure the fiducial marks are seen in the image. In in situ photography of the stem section, we place the leaf on the platform so that the latter is as close as possible to the stem without breaking the leaf. Focus and white balance are checked and changed as needed. A skilled team can photograph a leaf in approximately a minute, exclusive of travel of people or plants.

Data Collection and Quality Control. All plants are identified by a 15-character, 128-bit barcode that includes year, crop, family, row, and plant number. This barcode is scanned for each set of data recorded. Symbol Technologies' SPT 1800 barcode scanner running Portable Technologies Solution's TracerPlus/TracerPlusPro is used for the bulk of data collection. Occasionally paper forms pre-printed with barcodes are used for recording image numbers if the scanners are busy elsewhere. The data are later entered by scanning the forms or direct entry into a spreadsheet.

Several types of data relevant to the photographs are recorded: absolute and relative leaf numbers; plant identifiers; type of section; image number; photographer; and date and time. The

absolute leaf number is determined as soon as possible in the season by tagging fifth, tenth, and fifteenth leaves (the fifth while the coleoptile is still visible). In the 2007 season we used 9 in paper-covered, white, red, and blue twist ties, respectively, to mark the different leaves, loosely encircling the stem above that leaf and twisting them closed. (Usually marking the fifteenth leaf is not necessary.) We have found marking the leaves with twist ties, rather than ink spots, very significantly speeds up data recording and improves quality control. Relative leaf number is counted with respect to the first ear leaf (e0) and the tassel. We record the relation between absolute and relative leaf numbers for each plant in mutant lines when we collect other anatomical data on the plant.

During photography, the plant barcode, absolute leaf number, section, and image number are recorded. The first two are scanned in prior to leaf collection and retained in the scanner so that they can be reused for other images of that leaf. The image numbers are entered once they have been shot and checked. For ex situ photography, the fastest method was to use two scanners per photographic team, so that a different scanner was associated with the current and next leaves. We check the images by viewing them on the camera's display after each leaf, and only record the numbers of images we intend to use. This lets us re-shoot any poor sections before we move away from the plant or discard the leaf. The other fields of the image menu (photographer, camera, light, etc.) are set to the session's defaults or taken directly from the scanner's internal clock (date, time; scanners are synchronized to within a minute). The photographic details are collected in the EXIF data the camera bundles with each image.

To help ensure quality, each member of the photographic team has specific responsibilities. The photographer is responsible for directing leaf positioning, focus, lighting, and image quality. The leaf-holder is responsible for placing fiducial marks, checking leaf cleanliness, and aligning the leaf on the platform. In ex situ photography, this person also records the section and image data. The leaf collector is also responsible for gathering the plant and leaf data; during in situ photography, this job falls to the plant- or umbrella-holder. The other team members monitor the data collection and photography processes and point out errors and confusions. During a photographic session, the images are periodically uploaded to a laptop computer and visually checked for quality by the team. We do this early in the session to confirm settings, and at 1-2 other times to monitor quality and team fatigue.